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Understanding and Predicting Grain Nitrogen Concentration in Malting Barley

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Contents

Declaration

I hereby declare that this thesis was composed by myself and that the work contained therein is my own, except where explicitly stated otherwise in the text.

Eamon Nolan

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Lay Summary

Achieving grain nitrogen (N) concentrations in spring barley which are high enough to satisfy malting barley market requirements (1.52-1.84%) under Irish conditions has become difficult in recent years. The reasons for these low nitrogen concentrations are unclear, but one possibility is that conditions have favoured the growth of crops with a large yield potential effectively diluting the N accumulated in the grain. There is considerable interest amongst growers and maltsters in being able to forecast grain N concentrations from measurements made prior to harvest. This would enable growers to adjust crop management to increase grain N concentrations in crops at risk of not meeting malting specifications and would allow maltsters to plan their operations in advance of harvest. The aim of research conducted in this project was to investigate the scope for predicting grain N concentration from measurements of crop and soil characteristics made at ear emergence.

Field experiments were conducted in 2013 and 2014 at three site-seasons representative of those employed in malting barley production in Ireland. These experiments were designed to produce a large variation in crop growth and grain N concentration ranging from 1.0 – 2.5 % N and were used to identify crop characteristics closely related to grain N concentration. Grain N concentration was related to both N content and grain yield at harvest; these in turn were strongly associated with crop N content and biomass, respectively, at ear emergence. Statistical models using measurements of crop N content and biomass at ear emergence accounted for up to 80% of the variation observed in grain N concentrations suggesting that the models could be useful practical tools for predicting grain N concentration. The accuracy of predictions was tested using a separate set of data collected in 2015 from experimental plots and commercial spring barley crops representing several varieties grown over a range of sites. The accuracy was assessed by comparing values of grain N concentration predicted from crop measurements made at ear emergence with actual values measured at harvest. The best model gave accurate predictions when weather conditions were comparable to the long term average for the region. Further experiments showed that grain N

concentrations could be increased with N fertiliser applications at flowering. The research conducted here has shown that it is feasible to predict grain N concentration prior to harvest. After further testing, the system could be adopted by growers and maltsters to aid management.

Abstract

Grain nitrogen (N) concentration is a major quality criterion of malting barley for which there is a narrow range that producers must meet to satisfy market requirements (1.52 – 1.84 %). In recent years growers in Ireland have had difficulty producing grain with a high enough N concentration to meet these requirements using standard recommended agronomic regimes. The reasons for the lower than expected grain N concentrations are not known. There is interest from growers and maltsters in the development of a system to forecast likely grain N concentration from crop measurements made at or before flowering. A forecasting system would allow growers to identify crops at risk of falling below specification and to apply late N fertiliser if needed. It would also enable maltsters to plan grain intake and malting operations in advance of harvest. The aim of this project was to investigate the potential for predicting grain N concentrations of spring barley from crop and soil measurements made at ear emergence. The main objectives were to 1) investigate the relationships between measurements made at ear emergence and grain N concentration at harvest in order to identify which characteristics should be included as variables in multiple regression models to explain variation in grain N concentration, 2) to use the models with independent data sets to predict grain N concentration and test the accuracy of the predictions, 3) to quantify the recovery by the crop of fertiliser N applied at anthesis and its effects on grain N concentration and 4) to determine whether non-destructive techniques can provide estimates of crop growth and N content for use in the prediction models.

Field experiments were established with plots of spring barley (*Hordeum vulgare cv.* SY Taberna) at one site in 2013 and two sites in 2014 representative of those employed in malting barley production in Ireland. Fertiliser N applications were varied over the range 0 – 210 kg N/ha (with dressings split between sowing and mid-tillering) to provide a range of crop growth and grain N concentrations. In some experiments additional applications of N were made at anthesis to quantify effects on grain N concentration and seed rate treatments (150, 300 and 600 seeds per m^{-2}) imposed to test the accuracy of predictions of grain N concentration in crops of

varying canopy structure. Destructive samples were taken to determine total crop N content and canopy N distribution at ear emergence and harvest. Measurements of soil mineral N availability, ear numbers per m^{-2} and the number of spikelets per ear were made at ear emergence. Final grain yield and quality were also determined at harvest.

Grain N concentration is the quotient of grain N content and grain yield. Both grain N content and yield explained a significant amount of the variation in grain N concentration observed across sites and fertiliser N treatments indicating that estimates of both must be included in models to predict N concentration. Grain N content was strongly related to total crop N content at harvest ($P<0.001$; $R^2 = 0.96$), which in turn was related to canopy N content at ear emergence ($P<0.001$; $R^2 = 0.94$). Similarly, grain yield was strongly related to total crop biomass at harvest ($P<0.001$; $R^2 = 0.83$), which in turn was related to crop biomass at ear emergence ($P<0.001$; $R^2 = 0.88$). These results indicated that predictions of grain N concentration might be possible from measurements of crop N content and biomass at ear emergence and that the effects of variation in harvest index, nitrogen harvest index and post-anthesis N uptake on grain N concentration are likely to be negligible under normal agronomic conditions in Ireland.

Weather conditions in 2013 were unusually dry and estimates of soil moisture deficit and available water capacity indicated that the crop was water stressed. In 2014 weather conditions were close to the long term averages for the sites. Multiple regression models using canopy N content and biomass at ear emergence as explanatory variables accounted for 91% of the variation in grain N concentration when data from 2014 were used and 80% when data from both 2013 and 2014 were combined.

The models developed using data from plots sown at 300 seed per m^{-2} in 2014 were tested against independent data from plots sown at 150 seeds per m^{-2} in the same year and at the same sites to test the accuracy of predictions across plant populations and canopy structures. The models were also tested using data from experimental plots and commercial fields collected in 2015 to test the accuracy of predictions in a different year across a range of sites and varieties. Values of grain N concentration

predicted from measurements at ear emergence were compared with actual grain N concentrations measured at harvest. The accuracy of predictions was good with an R^2 of 0.80 and RMSE of 0.114 %N for the test across seed rates and R^2 of 0.80 and RMSE 0.220 %N for the validation in 2015 across sites and varieties.

In 2014, grain N concentrations were increased significantly by applications of additional N fertiliser at anthesis with apparent recoveries (increase in N content (kg) /kg fertiliser N applied) in grain averaging 50% over the range of application rates indicating scope for increasing grain N concentration in crops predicted to be at risk of not meeting malting specifications

Non-destructive measurements displayed significant relationships with N content and biomass at GS 59 across a combination of sites and seasons. However, issues in performance relating to instrument saturation were obvious and estimates never produced more accurate predictions of grain N concentration than destructive sampling**.**

The results show that grain N concentration of spring barley can be predicted with good accuracy from measurements of canopy N and crop biomass made at ear emergence when the weather conditions are comparable to the long term average for the region. As conditions of drought are rare in Ireland, the prediction models are a potentially valuable tool to aid crop management and post-harvest operations by growers and maltsters. Further testing will be needed before users can be confident in the reliability of predictions over years and a larger set of varieties.

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List of Abbreviations

aN	anthesis nitrogen
ANOVA	analysis of variance
BLUP	best linear unbiased predictor
cm	centimetre
dm	dry matter
e.g.	for example
ELADP	Ellipsoidal Leaf Angle Distribution Parameter
EU	European Union
g	gram
GAI	green area index
GNC	grain nitrogen concentration
ha	hectare
HI	harvest index
i.e.	that is
kg ha^{-1}	kilograms per hectare
kg	kilogram
km	kilometre
LAI	leaf area index
LTA	long term average
m	metre
m^{-2}	square metre
MGW	mean grain weight
mm	millimetre
N	Nitrogen
NDRE	Normalised Difference Red Edge
NDVI	Normalised Difference Vegetation Index
NH_4^+	Ammonium
NHI	nitrogen harvest index
Nmax	nitrogen rate for which maximum was achieved
NO_3^-	Nitrate

NUE	Nitrogen Use Efficiency
NU_pE	Nitrogen Uptake Efficiency
NU_tE	Nitrogen Utilisation Efficiency
PAR	photosynthetically active radiation
PAW	plant available water
PN	photosynthetic nitrogen
RMSE	root mean square error
RN	reserve nitrogen
RSA	root system architecture
S	site
SD	standard deviation
SMD	soil moisture deficient
SN	structural nitrogen
SPAD	soil plant analysis development
SR	seed rate
t ha^{-1}	tonnes per hectare
t	tonne
TGW	thousand grain weight

Symbols:

<	less than
>	greater than
%	percentage
+	plus
-	minus
°C	degrees Celsius
±	plus or minus

1 General Introduction

Barley (*Hordeum vulgare* L.) ranks fourth in global cereal production after rice, wheat and maize and is grown throughout the world on approximately 48 million hectares of land (FAOSTAT, 2013). Its wide geographical distribution reflects its relative tolerance to a range of growing conditions. It can be grown in climates ranging from the hot arid Middle East to the cool wet conditions of western Europe, at latitudes up to 70^{o}N (Grando and Gomez MacPherson, 2005) and at higher altitudes than most other cereals can tolerate (Fischbeck, 2002). Across this range, production systems differ in both their extent and intensity of production. For example, some of the largest producing nations such as the Russian Federation and Canada grow large areas of barley with relatively low yields in the range 2-3 t ha^{-1}, whilst others such as the UK and France produce higher yielding crops (~6 t ha^{-1}) using more intensive systems on smaller areas of land (Newton *et al.,* 2011). Over the last 50 years there has been a general decline in the area of barley grown; yet total global production has risen as a result of improvements in yield per hectare.

It is widely accepted that barley was one of the first crops to be domesticated, commencing in the Fertile Crescent around 8,000 BC, from its wild relative *Hordeum spontaneum* (Zohary and Hopf, 1993). It has been suggested that during domestication, naturally occurring forms with a less brittle rachis were selected because it enabled whole ears to be harvested without loss of spikelets (Newton *et al*., 2011). It was domesticated in the first instance for use as food, but over time its importance as a direct food source in human diets has declined. Direct consumption is now largely confined to the mountainous regions of Africa, Asia and South America where it can be grown more reliably than other cereals (Newton *et al.,* 2011). Elsewhere it has been developed to serve other purposes with grain being produced for animal feedstuffs, the malting, brewing and distilling industries and seed markets. On mixed farms straw is generally used as animal feed or for bedding, whilst on arable farms it may be reincorporated back into the soil. In developing countries, it may find additional uses such as roofing for dwellings. Ullrich (2011) stated that between 55-60% of total world barley grain production is used as animal

feedstuff, 30-40% is used for malting and brewing, approximately 5% for seed and only 2-3% for direct human consumption.

For grain to be acceptable for malting it must meet specific quality criteria. Typically these are a minimum germination of 98%, a moisture content of 12% (with possible price deductions if it exceeds 15%), a minimum size (90% retention on a 2.5 mm sieve) and admixture of foreign material of less than 2%. In addition the grain must be of a single variety and be relatively free from moulds and fungal metabolites. One of the most exacting specifications is the grain nitrogen (N) concentration; an N concentration of 1.52 – 1.84% (equivalent to a crude protein concentration of 9.5 – 11.5%) is required. Within this range the specification can differ depending on the end use of the malt. For brewing in the UK the requirement is 1.6 to 1.75% for ales and stouts, whilst for export and use in lager production it is higher, typically 1.7 to 1.85%. Barley for distilling, on the other hand, requires a lower N concentration of around 1.5%.

There is sound scientific reasoning behind the implementation of such a restricted and exacting grain N concentration set by maltsters. High grain N concentration results in a lower malt extract (Eagles *et al*, 1995; Howard *et al*, 1996), but it can also reduce the final product quality leading to cloudy beer. However, if the N (protein) concentration is too low, there may be insufficient enzyme activity to break down the starch during mashing.

In Ireland, barley makes up approximately 60% of the national cereal area, the majority (80%) of which is spring sown barley (Kennedy *et al.*, 2016). The relatively cool wet climate is conducive to the production of high yields. Over the years 2000 to 2009, average farm yields in Ireland were the second highest in the world at 6.6 t ha^{-1}, and in favourable years spring barley yields have exceeded 10 t ha^{-1} (Kennedy *et al*., 2016). There is a high demand for feed-barley in Ireland, but there is also a thriving malting market. However, in recent years the grain N concentration of spring malting barley in Ireland has been inadequate to meet malting barley market requirements despite growers following standard N fertiliser recommendations. For example, in a survey of grain nitrogen concentrations in commercial crops harvested in 2011, approximately 75% of samples failed to reach

the accepted minimum concentration (>1.52 nitrogen) (Figure 1.1) (Hackett, R. personal communication, 2012).

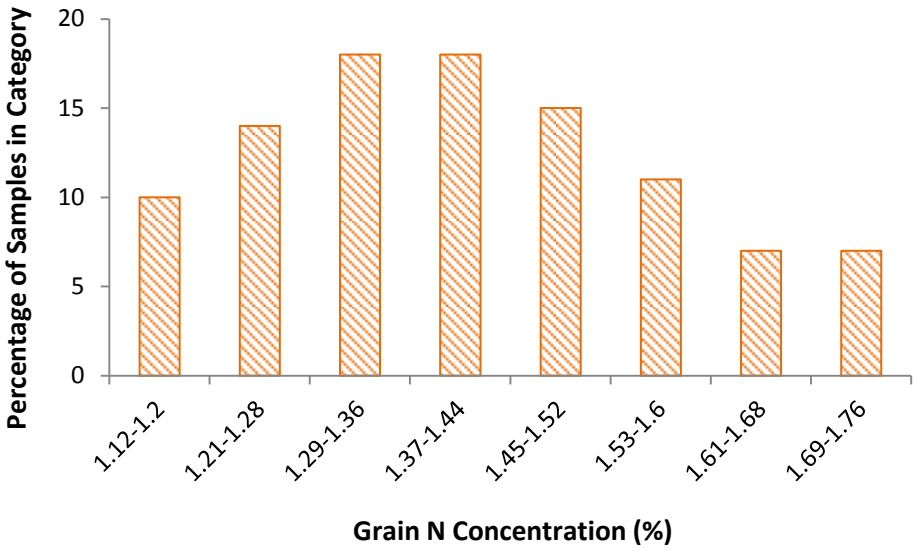

Figure 1.1. The percentage of samples in each category of grain N concentrations of commercial crops (2011). Approximately 75% of crops surveyed failed to achieve malting level specification for grain N concentration.

The reasons for this are unknown, but it may relate to the high yields being achieved, effectively diluting the concentration of N in the grain. Management regimes for growing malting barley generally result in lower yields than those for feed barley although this is usually compensated for by the higher price paid for malting quality grain. Failure to meet the grain N specification for malting therefore represents a significant financial loss to the grower. The consequences for maltsters are also significant because they can find themselves with an insufficient supply of high quality grain to meet their demand. There is strong interest amongst growers and maltsters in being able to forecast likely grain N concentrations early in the season. For growers it would enable them to adjust their N fertiliser management for crops that are predicted to be at risk of having an inadequate grain N concentration. For maltsters, an early forecast would allow them to plan their grain intake strategy and subsequent malting operations prior to harvest.

The overall aim of this project was to investigate the potential for predicting grain N concentration from measurements of crop and soil made pre-flowering. For a forecasting system to be of value and adopted in practice it must be accurate and reliable across different crops, sites and years. Ideally it should also be quick and easy for growers, agronomists and maltsters to use and require a minimal number of inexpensive measurements. To design a prediction system that will meet these criteria a good understanding is required of the N dynamics of spring barley crops, the factors that cause variation in grain N concentration (especially under Irish conditions) and the types of within-season measurements that are available.

2 Literature Review

2.1 Nitrogen

2.1.1 Nitrogen – Forms of Nitrogen in the Soil

Present in both organic and inorganic form, soil nitrogen is subject to seasonal and diurnal changes, in addition to the variable distribution in the soil profile (Miller and Cramer, 2004). Organic nitrogen in the soil is present in complex molecules, which are converted to ammonium by soil micro-organisms (mainly bacteria, fungi, protozoa, etc. (Foster, 1988)) via mineralisation. As there exists such a variety of organisms that are capable of mineralising nitrogen, the conditions required are not very specific, but certain conditions do enhance mineralisation. Temperature (Leirós *et al*., 1999) and moisture (Sierra, 2002; Cassman and Munns, 1980) have strong influences on mineralisation. Microbial activity is limited at near freezing and consequently increases with rising soil temperature, while dry soils restrict microorganisms due to water availability (Lewis, 1986). Soils with greater than field capacity water content tend to reduce the rate of nitrogen mineralization due to lack of oxygen or aeration where only anaerobic microorganisms are present (Stanford and Epstein, 1974) and the carbon/nitrogen ratio (Janssen, 1996) and soil pH (Fu *et al*., 1987) can also influence mineralisation rates. Clay content of the soil affects mineralisation rates (Deenik, 2006) with greater mineralisation rates achieved in coarse-textured soils with low clay contents and less mineralisation as the clay content increases.

Ammonium can then be oxidised via nitrite (NO_2^-) to nitrate (NO_3^-) during the nitrification process. The process of nitrification is impacted negatively by low soil pH, anaerobic conditions, soil water deficits and temperatures below 5°C and greater than 40°C (Lewis, 1986). Additionally, nitrate can be converted to nitrogen gases, via the process of denitrification. This process occurs when the oxygen becomes limited, nitrate concentration is high, soil water content is high, soil carbohydrates are available and temperatures are raised (Strong and Fillery, 2002). Up to 98% of the total nitrogen in soil is found in organic matter (including peptides, proteins,

amino acids, amino sugars and urea), which are mostly unavailable to plants, with the remainder in inorganic forms (and directly available to plants) (Miller and Cramer, 2004; Dechorgnat *et al*., 2011; Tischner, 2000; Schulten and Schnitzer, 1998) but soil organic nitrogen can be taken up by plants and can represent a proportion of total N uptake, under certain environments, such as acidic soils and low temperatures (Gastal and Lemaire, 2002). Both nitrate and ammonium are mobile in the soil (Maathuis, 2009), with one day diffusion distances in soil for nitrate and ammonium of 7.51×10^{-3} and 6.80×10^{-4} respectively; this illustrates why ammonium is less readily leached from the soil than nitrate. This is in contrast to organic N compounds, such as amino acids, which are less mobile and have a daily diffusion distance of less than 1 mm day^{-1}; there is research now suggesting that these forms can play a vital role as plant N sources (Miller and Cramer, 2008; Jämtgård *et al*., 2008).

2.1.2 **Factors influencing N uptake**

There are several factors that can impact on the uptake of N in the plant. It has been found that the presence of ammonium in uptake media reduces nitrate influx to the roots of crop species and into microorganisms within minutes (Glass, 2003). Furthermore there have been studies showing that the rate of uptake of both nitrate and ammonium vary immensely throughout the diurnal cycle (Glass, 2003; and references cited within). In an experiment by Clement *et al.* (1978), peak uptake occurred in the late afternoon or during the first hours of night, while minimum uptake occurred before midday. Such changes are attributed to the diurnal changes in irradiance levels. Therefore, both diurnal oscillations and total irradiance can influence greatly nitrogen uptake. This impact (a general decline) on N uptake is influenced by the progressive depletion of the non-structural carbohydrate pool in the shoot as well as the concentration of carbohydrates in roots (Rideout and Raper, 1994). Although a relationship exists between them, crop nitrogen uptake is ultimately governed by crop growth rate, of which temperature is a key parameter. Many crops encounter growth limitations or restrictions as a result of low temperatures. Therefore the plant's demand for nutrients is reduced for, at times,

considerable periods of the year, resulting in reduced nutrient uptake and increased risk of loss to the environment. Such effects of temperature do not just affect N uptake via growth rate; there are consequences of temperature on the transport systems within the plant (Glass, 2003). The effects of temperature upon the nitrogen transport systems of plants can be seen in the work of Glass *et al. (*2002) and Wang *et al. (*1993). An internal plant regulatory mechanism has been established as responsible for the control of N uptake in plants. Under steady state conditions, a pool of amino-N cycles in the plant, between the shoot and the root, reflecting the above ground demand for nitrogen and regulating soil N uptake (Touraine *et al*., 1994).

2.1.3 **Effect of soil management (current and past) on soil N pools**

There have been several studies carried out examining the effect of cropping rotations on soil nitrogen mineralisation potential. Such work has shown that the selection of cropping rotations can affect soil nitrogen mineralisation, and rotations that include a legume crop can result in increased net nitrogen mineralisation (Carpenter-Boggs *et al*., 2000; Deng and Tabatabai, 2000). The method of crop establishment has been shown to provide a small amount of influence on the mineralisation of soil N. In work conducted by El-Harris *et al. (*1983), data was presented that suggested nitrogen mineralisation following mouldboard ploughing was uniform in the 0-15cm soil layer, but mineralisation rates were greater in the 0-5cm depth and less in the 5-15cm layer after chisel ploughing and no-till establishment practices compared to ploughing. Although a specific soil layer effect was seen, there was essentially no effect of tillage practice on nitrogen mineralisation. The level of nitrogen mineralised from organic matter decreases, as the period of time a soil is in tillage increases (Herlihy, 2001). This reduction in soil organic matter can be attributed to a number of agricultural practices, especially cultivation of the soil; each time the soil is tilled, especially inversion tillage, oxygen is incorporated into the soil, where the decomposition of organic matter and the liberation of carbon are aerobic processes. The oxygen stimulates the action of soil microbes, which feed on organic matter. Other factors such as the removal of crop

residues and the absence of farm yard manure applications can also play a part in the decrease. When green manures are incorporated into the soil they induce a flush of nitrogen mineralisation (Mengel, 1996); it has been shown also that the degree of mineralisation increased with the rate of application (Mohanty *et al*., 2011). This is due to the C:N ratio of the organic material added to the soil. It is the magnitude of the ratio which determines the rate of decomposition of the organic matter and the subsequent mineralisation (release) or immobilisation of soil nitrogen. When the nitrogen content is relatively high, the microorganisms rapidly release nitrogen when they decompose the organic matter. When the carbon content is high (greater than 30:1), indicating a low nitrogen content, the mineralisation process is slow. In order for the organisms to break down a high C:N material, inorganic nitrogen is removed from the soil through the process of immobilisation. If the material has a high enough C:N ratio all of the inorganic nitrogen can be removed from the soil for a considerable amount of time (Braakhekke *et al*., 1993). When farmyard manure (FYM) is applied and incorporated into the soil, it can result in the immobilisation of nitrogen derived from the manure itself and to a greater extent, the soil (Thomsen, 2005), irrespective of the rate of application (Mohanty, *et al*., 2011), due to the C:N ratio of the material and its high carbon content.

Timing of the application of farmyard manure with regard to spring sown crops is critical, concerning nitrate leaching losses and crop N utilization. The recovery of nitrogen by spring barley from farmyard manure that was applied in September was just 8% of total N, while this increased to 17% for FYM that was applied later, in December or March. It was found that the application method could not compensate for the higher potential of N leaching losses which occur after autumn application (Thomsen, 2005). FYM is made up of bedding material (straw), with faeces and urine and has a high C:N ratio, immobilisation of N derived from both the manure itself and from the soil may be more pronounced. The agronomic practice of the incorporation of organic matter, such as straw, into soil results in the immobilisation of inorganic nitrogen (Mengel, 1996). In an experiment conducted by Powlson *et al. (*1985) examining the effect of straw incorporation on N uptake by winter wheat, it was found that straw incorporation reduced nitrate loss from the crop-soil system compared to non-incorporated straw. It provided little effect on N uptake by the crop,

as the straw immobilised inorganic nitrogen that would have otherwise been lost through leaching. Additionally, only 12% of the nitrogen from the straw was recovered, while 78% remained in the soil after one year. Furthermore, the deeper that the organic matter was incorporated into the soil (in this experiment, 0-30cm), the larger the impact of immobilisation that occurred, as more mineral nitrogen is available, and resulted in less leaching (Garnier *et al*., 2003).

It was found that during the first winter, incorporated straw immobilised more than 10kg N/ha, but this nitrogen was later released at a constant rate (Christensen, 1986). The rate of nitrogen immobilisation is influenced by the timing of straw incorporation and is greater when straw incorporation takes place directly prior to spring applied mineral fertiliser (Bohgal *et al*, 1997). Studies have shown that the application of N fertiliser leads to 10-20% of the aforementioned fertiliser being assimilated by soil microbes and therefore, is contributed to the organic soil nitrogen pool (Schnier *et al*., 1988). This nitrogen pool is of use to the following crop (Mengel, 1996), although only a small proportion of it represents that taken up by subsequent crops after the crop in the experiment (Hart *et al.,* 1993).

Although inorganic forms of nitrogen, nitrate and ammonium are considered to be the two most abundant compounds taken up by plants, organic forms such as amino acids, peptides and small proteins are also important sources. The preferred form of nitrogen that is taken up by a plant is dependent on the plant's adaptation to soil conditions. Plants or crops that have adapted to soils with low pH and anaerobic reducing conditions are predisposed to taking up ammonium or amino acids, in contrast to plants adapted to soils with higher pH levels and aerobic conditions, which favour the uptake of nitrate (Masclaux-Daubresse *et al*., 2010).

But it has been shown that when equimolar concentrations of ammonium and nitrate are supplied, ammonium is taken up as the preferred nitrogen, especially when nitrogen availability is low (Gazzarrini *et al*., 1999). This favouritism for ammonium uptake over nitrate uptake is emphasised as temperatures declines; ammonium uptake continues under 5°C while nitrate uptake ceases. This may be due to the lower energy requirements associated with the uptake and assimilation of ammonium compared to nitrate (Macduff and Jackson, 1991). In spite of the fact that plants can

absorb ammonium in preference to nitrate under some conditions, they are unable to accumulate ammonium ions to such high concentrations in the tissue as toxicity can occur, particularly in the absence of nitrate (Britto and Kronzucker, 2002). Ammonium toxicity can present several symptoms in a majority of, if not all, plants when cultured exclusively on it as an N source (Gerendas *et al.,* 1997). Barley is reported as very sensitive to ammonium toxicity, with two dramatic symptoms of chlorosis of leaves and a general suppression of growth (Kirkby and Mengel 1967; Kirkby 1968). It has been shown that yield penalties from 15 to 60% can be attributed to ammonium toxicity (Woolhouse and Hardwick, 1966) while death of plants can also result (Gigon and Rorison 1972; Pearson and Stewart 1993). While other symptoms such as lowering of root: shoot ratios, changes in plant roots including a decrease in the fine: coarse root ratio, additional stimulation of root branching and reduced mycorrhizal associations can be experienced.

Strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil' as defined by Kuzyakov *et al. (*2000) describes the priming effect. It can be initiated by the integration of organic or mineral fertiliser to the soil, exudation of organic substances from the roots, mechanical processes or the drying and rewetting of the soil. Essentially, the priming effect results in substantial volumes of C, N and other nutrients to be either released or immobilised in a short period of time. In the case of nitrogen, the priming effect is additional soil nitrogen that is taken up by plants after the application of mineral N fertiliser, compared with that of untreated plots (Jenkinson *et al.,* 1985). Kuzyakov *et al. (*2000) provided a detailed review of the mechanisms which result in the priming effect. These include application of mineral N fertilisers, easily available organic substances, soil drying and wetting, plant rhizodeposition, soil physical impedance such as cultivations, etc.

2.1.4 **Nitrogen Uptake Mechanisms**

The fundamental principle governing the uptake of nutrients is the necessity of the ion to be adjacent to or in proximity of the root. The appropriate location of the ions can be achieved by root interception, mass flow or diffusion. Root interception is the

growth and development of a root through a medium where it can accidently contact nutrients, thus facilitating ion uptake (Oliver and Barber, 1966). There is now evidence suggesting that roots may be able to sense certain nutrients and trigger root growth in the direction of the nutrient pool (Robinson, 1994). Mass flow occurs when available solutes (i.e. those not held by soil fractions) present in the soil solution flow to the root of the plant as water is taken up. The amount of nutrients that are moved to the plant root by this process is governed by the amount of water taken up by the plant and the concentration of nutrients in the water (Oliver and Barber, 1966). As plants take up nutrients, the roots deplete the immediate soil solution of ions. This creates a concentration gradient for the nutrients to diffuse through the soil solution, moving from a zone of high concentration to the depleted area adjacent to the soil solution; this is known as diffusion (Jungk and Claassen, 1997).

From the root surface, water and minerals can move by three major pathways across the root cortex toward the plant's vascular system. Transport can occur via the apoplast, the symplast or a trans-membrane (vacuolar) pathway (Dechorgnat *et al*., 2011). Apoplastic transport entails movement of water and solutes across the cortex, through the cellulose cell wall and intercellular spaces. This cellulose wall is permeable and non-selective. In plants lacking a hypodermis, water and minerals can be transported along this pathway until they reach the endodermis. Here, the pathway is blocked by the substance suberin, which is located in the cellulose cell walls of the endodermis; this is what forms the Casparian band or strip. At this junction, the solute must cross the plasmalemma and move into the stele via the symplast or vacuolar pathways. The symplastic pathway involves movement of solutes through the cytoplasm of cells and from cell to cell via the plasmodesmata while transmembrane transport occurs when the solute enters and exits the vacuoles en route through the protoplast. Essentially, all three transport systems may operate in conjunction with one another for the transport of mineral nutrients across the root cortex.

2.1.5 **Nitrogen Uptake**

Nutrient concentrations in roots of plants can be much greater than those of the external solution – thus uptake from external solution requires both a source of energy and selective transport across the plasma membrane of root cells. Transport across the plant membranes is facilitated by transmembrane proteins known as ion transporters. The uptake of N by the plant can be achieved by a number of uptake systems (Miller and Cramer, 2004). Research has shown that plants possess both high affinity transport systems (HATS) and low affinity nitrate transport systems (LATS), which function at varying external concentrations of nitrate (Maathuis, 2009). The high-affinity transport systems (HATS) function when nitrate in the soil is at a low concentration (<1 mM) and low-affinity transport systems (LATS) operate at high concentrations (>1 mM) (Dechorgnat *et al*., 2011). It is also now understood that there are two high affinity transport systems which are distinctly different. The first is the constitutive high affinity transport system (CHATS) which displays low values of V_{max} (the maximal speed of activity of the enzyme) and K_m (the concentration of substrate required to produce 50% of the Vmax value). The second is an inducible high affinity transport system (IHATS) which displays higher K_m and V_{max} values and are induced between hours and days, following exposure of roots of N-deprived plants to nitrate (Glass and Siddiqi, 1995). Constitutive low affinity transport systems (LATS) can significantly contribute to nitrate uptake at external concentrations above 250μM, but are unable at concentrations as high as 50mM (Crawford and Glass, 1998). Okamoto *et al. (*2006), suggested that the LATS is also divided into two classes, including a constitutive low affinity system (CLATS) and a nitrate inducible low affinity system (ILATS). With regard to ammonium uptake, it has been established that both a HATS and a LATS exists in the plant roots. Both appear to be constitutive and are not induced by ammonium (Glass *et al*., 2002).

Ion uptake for plants can be very energy demanding, especially during periods of rapid growth. White (2012) suggested that over one third of the total respiratory energy cost (ATP consumption) is utilised in seedlings, to conduct ion uptake. But this percentage reduces as the plant matures to support ATP and energy demands for

biomass growth and maintenance. When high concentrations of ions (such as NH_4^+ and NO_3) are present in the rhizosphere solution, there is a high rate of futile cycling present in the plasma membrane of the roots. Additionally, nitrogen cycling in the roots can occur, where influx of nitrogen into the roots can be followed by the efflux of nitrate, ammonium and amino acids back into the soil solutions. This process can occur especially when nitrate levels are high (Kronzucker *et al*., 1999), and has been shown to cease completely a number of hours after nitrogen deprivation (van der Leij *et al*., 1998). It has been suggested that this nitrogen efflux is used as a method of sensing nitrate availability in the soil (Miller and Smith, 2008). As this concentration increases, the rate of cycling increases; resulting in an extensive energy cost for the root respiratory system.

2.1.6 **Nitrogen Metabolism**

Nitrogen in the form of nitrate must be reduced to ammonium ($NH4^+$) before it can be integrated into the organic components of plant tissues. The course of nitrate reduction in plants occurs through nitrate reductase and nitrite reductase. Nitrate reductase catalyses the two-electron reduction of nitrate to nitrite (NO_2^-), while nitrite reductase transforms nitrite to ammonium in a six electron transfer process (Crawford and Arst, 1993). The nitrite created by the process of nitrate reductase is transported to the chloroplast (plastids in roots) where nitrite reduction converts nitrite to ammonium (Kant *et al*., 2011). Although the majority of plants can reduce nitrate in the roots and shoots, the ratio of nitrate reduction that takes place in the roots and shoots is determined by a number of factors including species, age and nitrate supply. In environments with low nitrate supply, a greater proportion of nitrate reduction occurs in the roots, while in situations of high nitrate concentrations, the roots' ability to accommodate such nitrate reductase is restricted and therefore a greater amount occurs in the shoots (Andrews, 1986). The energy requirement for nitrate reduction and assimilation is considerable and can influence the selected site of reduction (Gavrichkova and Kuzyakov, 2010). One mole of nitrate needs 15 moles of ATP for reduction processes in the roots, while ammonium requires only 5 moles ATP per one mole. (Salsac *et al*., 1987). With respect to

barley, up to 23% of root respiration energy is utilised for nitrate reduction, compared to 14% for ammonium (Bloom *et al.*, 1992).

Ammonium can be derived not only by root uptake from the soil, but is produced by nitrate reduction, photorespiration, senescence induced nitrogen remobilisation and, in leguminous plants, through N_2 fixation (Joy, 1988). Regardless of the original source of ammonium or the site of assimilation (roots, root nodules or leaves), two essential enzymes are employed; glutamine synthetase (GS) and glutamate synthase (GOGAT – glutamine-oxoglutarate aminotransferase). The majority of ammonium sourced from ammonium uptake, N_2 fixation, photorespiration and nitrate reduction, that is assimilated, is done so via the glutamine synthetase – glutamate synthase pathway (Hawkesford *et al.*, 2012; Tischner, 2000) and is ultimately used to produce all other proteinous amino acids. Low molecular weight organic compounds are used as intermediates for inorganic nitrogen assimilation and the synthesis of high molecular weight compounds. These low molecular weight organic compounds are also significant in the transport of nitrogen from source organs to sink organs and additionally, generate reserves of nitrogen during periods of high availability (Hawkesford *et al*., 2012)

2.1.7 **Nitrogen loss**

Considerable levels of nitrogen loss can occur from plants, and it is accepted that a significant proportion of this loss is incurred via ammonia volatilization from plant leaves (Mattsson and Schjoerring, 1996). However, both emission and deposition can take place. The exchange of gaseous NH_3 between plants and the atmosphere is governed by gradients existing between the substomatal cavities in the plants leaves and that of the atmosphere. When the concentration in the atmosphere is less than that of the leaves, ammonium emissions or loss occurs; while if the concentration in the atmosphere is greater than that of the leaves, deposition can arise (Schjoerring *et al*., 2000). A balance whereby neither loss nor deposition takes place is referred to as the compensation point, where both processes balance and result in zero net flux of

NH_3 (Farquhar *et al*., 1980). NH_3 emission from the leaves is most common, as their concentration is generally greater than the atmosphere. The amount of NH_3 which can be lost varies according to a number of factors including plant species, nitrogen status of plant and soil and the environmental climate conditions. Emissions can range in value from less than 10 kg NH_3 N/ ha to greater than 70 kg NH_3 N/ha, and can account for up to 5% of a plants shoot N content (Schjoerring *et al*, 2000).

Additionally, nitrogen cycling in the roots can occur where influx of nitrogen into the roots can be followed by the efflux of nitrate, ammonium and amino acids back into the soil solutions. This process can occur especially when nitrate levels are high (Kronzucker *et al*., 1999), and has been shown to cease completely a number of hours after nitrogen deprivation (van der Leij *et al*., 1998). It has been suggested that this nitrogen efflux is used as a method of sensing nitrate availability in the soil (Miller and Smith, 2008).

2.1.8 **Measurement of N uptake**

The measurement of nitrogen uptake is a fundamental measure of crop science. There are a number of methods that can be used to calculate it. The difference or indirect method (non-isotopic) calculates the level of applied N that is taken up by a crop by estimating the difference in total N uptake per unit of N applied between fertilised and unfertilised plots. The isotope-dilution or direct method calculates the amount of fertiliser nitrogen taken up by a crop by estimating the total N uptake and N isotope-ratio analysis of plant materials from fertilised treatments (Hauck and Bremner, 1976).

The advantages of isotope-dilution over the difference method are a consequence of the ^{15}N tracers which serve to differentiate between fertiliser N and non-fertiliser N; thereby allowing the calculation of fertiliser N uptake and fertiliser N uptake efficiency (Torbet *et al*., 1992). Additionally, it eliminates the possibility that total N uptake is a result of fertiliser N uptake (Sandrock *et al*., 2005). But a disadvantage is that it underestimates fertiliser N uptake when applied fertiliser ^{15}N is immobilised in

substrate organic matter and non-labelled substrate N is released for plant uptake (i.e. as a result of pool substitution) (Sandrock *et al*., 2005; references cited within). Furthermore, nonisotopic N uptake calculations provide consistently higher N uptake results than the isotope-dilution method.

2.2 Roots

2.2.1 Function and Structure

The principle functions of the roots of plants are the acquisition of water and nutrients and to provide anchorage for the plant in the soil. The optimum distribution of root length depends mainly on the distribution of water and nutrients in the soil profile. For example, the roots required by a plant to attain water that is deep in a soil in a dry season or environment, would need to be long; whereas if water and nutrient availability are lavish, then smaller root lengths may be acceptable (Bengough *et al*., 2006). One of the greatest influencing factors to the capacity and efficiency of nitrogen uptake is the number, size and location of root hairs. Fine roots provide a higher surface area to volume ratio than thick roots and additionally require less C for construction however, may have higher maintenance costs (Miller and Cramer, 2004). There are three major processes which affect the root structure and architecture of a plant. Initially, the primary root meristem undergoes cell division, enlarging the root. Followed by lateral root formation, that increases the exploratory capacity of the root system. Finally, the formation of root hairs increases the total surface of both primary and lateral roots. Any alterations to these processes can influence a crop's capacity to grow and develop in nutrient resource limiting soils (López-Bucio *et al*., 2003). The uptake of nitrogen to sustain the growth and development of a plant is heavily influenced by the root system. The species-specific root size and architecture is determined not only by the pattern of branching, but by physical, chemical and biological processes and factors (Miller and Cramer, 2004). Such stresses may occur continually or varyingly, depending on the location of the root and the prevailing conditions (Bengough *et al*., 2006).

2.2.2 **Chemical**

The effects of nutrient supply can provide a robust effect upon the growth, morphology and distribution of roots and nitrogen is a major influential example of such nutrients. Research has shown that with increasing nitrogen supply both shoot and root growth increases, but this effect is not equal on both plant fractions. Shoot growth is magnified to a larger extent than root growth from nitrogen application; and this thereby reduces the root: shoot ratio with incremental nitrogen applications.
Nevertheless, such a reduction in the root: shoot ratio does not impact upon the source sink ratio, as the roots exhibit a greater degree of branching and produce finer roots resulting in an increased surface area (i.e. source) to match shoot growth (demand). Additionally, it has been found that this phenomenon occurs to a greater extent with ammonium over nitrate as nitrogen sources (Marschner *et al*., 1986). Interestingly, it has been shown that if a plant's root system is curtailed by nutrient deficiency, other parts of the root system can adapt and increase to compensate (Bingham *et al*., 1997; Malamy and Ryan, 2001). On the other hand, root: shoot ratio decreases when nitrogen is limiting. This is a result of starch accumulation in the leaves and a further increase in the photosynthate translocated to the root (Rufty *et al*, 1988). Furthermore, it has been indicated that root nitrogen availability controls signals which sequentially determine the demand for growth and thereby the control of carbon allocation (Miller and Cramer, 2004).
Plant root growth is impacted little to none within the soil pH range of 5-7.5. At high soil pH levels, growth is known to be inhibited either directly or indirectly. Such high pH levels can impact upon the chemical species present in the soil solution and also the electrochemical gradient and solute transport (White, 2012). Ammonia toxicity is also highly associated with high pH and can severely inhibit root growth (Schenk and Wehrmann, 1979). Conversely, root elongation is inhibited in low soil pH due mostly to aluminium (Al) monomeric activity, and therefore creates aluminium toxicity. Furthermore, Al hinders calcium (Ca) uptake (Osaki *et al*, 1997), and it is known that in the absence of Ca, root growth ceases within a few hours as it is involved in both cell division and cell elongation (Burstrom, 1968). But, possibly

more importantly, Ca is involved in guarding root growth from the stress of low pH (Lynch *et al*., 2012).

Plants have high rates of respiration and consequently have high oxygen demands. The transfer of gases between soil and the atmosphere (gas diffusion) occurs in air filled pores, as it is approximately 100 times faster than in water. However, there are a number of species (such as rice) that have adapted to waterlogged conditions and therefore internal diffusion of oxygen occurs to meet requirements, although in non-wetland species (mesophytic), this internal diffusion capacity is insufficient to satisfy the demands of large root systems (Lynch *et al*., 2012). Interestingly, although the total critical concentration that affects root growth differs between varying species, in most mesophytic plants growth is not impacted upon even when oxygen concentrations reach 15-10% (Geisler, 1967). Additionally, as soil oxygen levels decrease, carbon dioxide levels increase, with an associated increase in ethylene concentration (Jackson, 1990). The concentration of carbon dioxide (CO_2) in the soil atmosphere increases with depth and reaches a maximum in summer when both root respiration and soil microorganisms are also high (Nakayama and Kimball, 1988).

2.2.3 **Physical**

In the process of root growth, roots must penetrate the soil profile by exerting forces sufficient to displace such soil particles, unless the growth of the roots follows the path of continuous cracks or voids in the soil. The equal and opposing force exhibited by the soil in response to root growth is referred to mechanical resistance or impedance (Bengough *et al*., 1997). The strength of a soil increases as the soil dries; and significant increases in strength can occur on various soil textures including predominantly sandy, silty or clayey soils (Bengough *et al*., 2006). Bulk density consequently can have a large impact on root growth. Research conducted by Goodman and Ennos (1999) demonstrated that soils with a low bulk density revealed significantly lower penetration resistance than that of high bulk density soil, essentially encouraging root growth. Finally, soil compaction usually does not occur in a uniform pattern regarding the depth or area in a field. Furthermore, such

compacted zones can restrict or inhibit a plants' ability to access water and nutrients in the aforementioned regions (Taylor and Brar, 1991). Bingham and Bengough (2003), found that when the different regions of the same root system of barley are exposed to contrasting soil conditions, such as locally compacted soil produced by a tractor wheel, or a plough pan; a compensatory adjustment of root growth occurs in the region of no/lesser compaction (in contrast to wheat). Although some research showed that, in the scenario of root growth being altered (or partially altered) by soil compaction, above-ground growth may be unaffected if the plant can acquire sufficient nutrients and water (Taylor and Brar 1991), while conflicting results by Bingham and Bengough (2003) show that even in the presence of compensatory adjustments, shoot growth is reduced when part of the root system is in the compacted soil.

Root growth is governed by cell turgor, and therefore, it must overcome these impedances and also the frictional force exerted along the exterior of the root (although this is usually low as roots exude or secrete mucilage to ease friction) (Bengough *et al*., 1997). Once roots come across a hard region within the soil profile, cell elongation is reduced, but radial growth increases. The rate of cell elongation slows, while the cell walls stiffen in the direction of growth; thus resulting in an increase in penetrating force of the root (Bengough *et al*., 2011).

The response of plants to insufficient water availability is an increase in biomass allocation to roots at the sacrifice of shoot growth and this thereby results in increased water capture and decreased water use. Plants increase root exploration of the soil profile in low soil water content environments, in search of the greatest water source. As discussed previously, as soils dry, the strength of soils increase with an associated increase in impedance; additionally, available water is usually located in the deeper soil layers and plants concentrate upon root growth to reach water reserves or sources. Essentially, plant roots that grow in search of water sources are faced with increasing impedance of the drying soil, while roots of plants with sufficient or adequate soil water content can continue elongating, resulting in deeper rooting (Lynch *et al*., 2012).

Similar to other traits of the soil profile, soil temperature can vary significantly with depth and time. The temperature of the uppermost surface layers fluctuate greatly in response to altering air temperature, irradiance and radiant heat transfers, while temperatures are more constant at greater depths. Although temperature influences root growth, the optimum for growth varies according to species, but is higher for shoot growth (Lynch *et al*., 2012). There is limited information regarding the effects of high soil temperatures on root growth, but it was shown by Huang *et al. (*1991) that in wheat as soil temperatures increased the root's ability to conduct water decreased. While at low soil temperatures, root elongation is reduced (Pahlavanian and Silk, 1988), and branching (Gladish and Rost, 1993) and root respiration are also reduced (Covey-Crump *et al*., 2002).

2.2.4 **Manipulating root growth**

Root distribution can be manipulated via the specific placement of fertiliser. Research has revealed that in water limited environments, the deep placement of fertilisers can provide yield benefits to crops. This result is mostly associated with the specific deep placement of fertiliser in the subsoil, below the dry top soil layer, where water may still be available late in the season. This can encourage deeper and prolific root growth in that region of soil (Ma *et al*, 2009).

The type of cultivation method practised can influence root growth. Work carried out on barley (*Hordeum vulgare* L.) examined the effect of subsoil tillage, minimum tillage and no-tillage operations on root growth. It showed that, although no-tillage resulted in the highest penetration resistance of all three treatments, root length density was also the greatest; suggesting that no-tillage provided favourable conditions for root growth in a well-structured soil. In the same experiment, the effect of crop management, or more accurately, crop rotation was also studied (Lampurlanés and Cantero-Martínez, 2003).

2.2.5 **Root development: Pre- and Post- Anthesis**

Conditions which encourage N mineralization are prominent in the upper layers of soil, making the nitrogen concentration in that region greater than lower level layers. Although, as Gastal and Lemaire pointed out, this contrasts with the crop's N uptake ability depending on rooting depth. The rooting depth of plants greatly influences a crop's capacity to intercept nitrate in periods of leaching and thus aid in the protection of the environment (Gastal and Lemaire, 2002). Root system architecture (RSA) is particularly important for soil resource acquisition by allocating root foraging to soil areas with the largest resources (Lynch and Brown, 2012). It is not just the rooting depth of mature crops that is important regarding the uptake of nitrogen, but also the rate that the roots of seedlings develop at depth; especially crops which have an early development phase during winter, when leaching can often occur (Gastal and Lemaire, 2002).

2.3 **Plant N**

2.3.1 **Leaf N and leaf photosynthesis**

The process of photosynthesis is fundamental to crop production. It is a highly regulated, multistep process, that involves the harvest of solar energy, transfer of excitation energy, energy conversion, electron transfer from water to $NADP^+$, ATP generation and a series of enzymatic reactions that assimilate carbon dioxide and synthesize carbohydrate (Tanaka and Makino, 2009). Nitrogen (both nitrate and ammonium) are assimilated by the crop and used to build the photosynthetic tissues containing large quantities of photosynthetic proteins (mostly Rubisco) and the structural nitrogen in supporting tissues and vascular connections of the shoot system (Lemaire and Gastal, 1997). N is involved in the functioning of meristematic tissues and photosynthesis and it is also used as a determinate of quality of harvested organs regarding nitrogen concentration. Up to 75% of the reduced N found in the leaves of cereal crops located in the mesophyll cells (mainly as Rubisco), and is involved in photosynthetic functions (Bertheloot *et al*., 2008).

2.3.2 **Nitrogen distribution between leaves of the canopy**

The photosynthetic capacity of a crop is governed by the total amount of leaf area and leaf nitrogen and on the vertical distribution of leaf area and nitrogen throughout the crop canopy (Aerts and De Caluwe, 1994). Due to the pattern of canopy growth and development, there are strong gradients of light intensity experienced within the canopy, resulting in nitrogen concentration gradients towards the top of the canopy (especially where stand density is high) (Hirose and Werger, 1987). Furthermore, as plant canopies are subjected to varying degrees of light intensity, such as shading by upper leaves, etc., nitrogen concentrations are not uniform within the plant (i.e. leaves at different positions in the canopy contain varying concentrations of nitrogen) (Aerts and De Caluwe, 1994; Gastal and Lemaire, 2002). Hirose and Werger (1987) and Hirose *et al. (*1989) found that nitrogen concentration increases in the plant canopy from the bottom to the top, but furthermore, that the pattern basically follows the pattern of light distribution in the canopy. The photosynthetic capacity of a plant is also impacted by leaf age, with increasing age resulting in decreasing photosynthetic activity (Field and Mooney, 1983). Canopy construction or structure provides a large influence on nitrogen concentration within the canopy, with plants that possess relatively horizontal leaves (dicot species) exhibiting steeper nitrogen gradients within the canopy than those plants with more erect leaves (Anten *et al*., 1995). Additionally, the level of soil nitrogen availability can vary throughout the growing season and consequently can impact upon leaf nitrogen concentration as leaf production is continuous, thereby further contributing to non-uniformity of canopy nitrogen concentration (Gastal and Lemaire, 2002). N supply can influence considerably the leaf nitrogen concentration of canopies (Gastal and Lemaire, 2002). It has been shown that most plants react to increasing nitrogen supply or availability by increasing leaf nitrogen concentration (Gulmon and Chu, 1981).

2.3.3 **Impact of leaf N distribution and photosynthesis**

Leaves require progressively less nitrogen to achieve maximum carbon assimilation potential descending from the top of the canopy to the bottom as a result of the reduction in irradiance levels (Gastal and Lemaire, 2002). Hirose and Werger (1987) found that plant canopies that displayed the greatest nitrogen content per unit area, in the regions of the most irradiation, demonstrated an advantage in terms of their photosynthetic capacity over canopies with uniform nitrogen distribution throughout. Additionally, it was discovered that such a pattern of nitrogen distribution mimics the pattern of light distribution (Hirose *et al*., 1989). This agrees with the suggestion of Field and Mooney (1983) regarding the ideal that canopy photosynthesis would be maximised if nitrogen preferentially allocated leaves to those receiving the highest illumination. Although it has been shown that leaf nitrogen distribution in most canopies closely approaches the optimal distribution in terms of maximising whole canopy potential carbon gain (Werger and Hirose, 1991), Hirose and Werger (1987) found that actual plant canopy nitrogen allocation resulted in over 20% increase in photosynthetic performance than that of uniform nitrogen distributions, while it achieved just 4.7% under that of the optimal allocation of canopy nitrogen. The distribution of leaf nitrogen inherently influences leaf and canopy photosynthesis which govern radiation use efficiency (RUE). Gastal and Lemaire (2002) provided a detailed review of the interrelationships of leaf N distribution. Ultimately, the effect that nitrogen has on crop growth via its effect on leaf growth (light interception) is more important than its effect on photosynthesis. However, nitrogen affects crop growth owing to its impacts on both leaf growth (light interception) and leaf photosynthesis; it is a consequence that there is somewhat a balance met between the allocation or distribution of nitrogen in the plant to maintain leaf nitrogen (and functions associated, including photosynthesis) in present plant fractions and the creation of new plant material (Sinclair and Horie, 1989).

2.3.4 **Crop N and leaf growth**

It has been demonstrated that the supply of nitrogen to plants increases leaf area and thus crop canopy to a greater magnitude than that of leaf and canopy photosynthesis (MacDonald *et al*., 1986). In grasses such as barley (Shi *et al*., 2013), this increase in leaf area of canopy is due to the expansion of individual leaves and on branching or tillering, while N supply provides a minor impact upon the rate of leaf appearance and the duration of leaf expansion (Gastal and Lemaire, 2002; and references cited within). Although it is known that N supply alters cell division and cell elongation rates, there are contrasting reports on the effect of nitrogen supply. Some experiments show that nitrogen stimulates cell production rate but affects the final cell length negligibly, whereas others found that nitrogen supply increased cell elongation rate but decreased duration of cell elongation (Fricke *et al*., 1997, and Gastal and Nelson, 1994, respectively). Essentially, the supply of N on leaf expansion is due to effects on cell production rather than cell elongation rate (Gastal and Lemaire, 2002).

2.3.5 **Contribution of plant parts to grain nitrogen**

The level of research work carried out into the contribution of various plant fractions towards grain N in barley is modest in comparison to wheat. As crops develop, the leaf to stem ratio diminishes (Bélanger and Richards, 2000) and therefore the allocation of nitrogen and carbon to leaves is reduced in favour of accumulation in other plant fractions, such as the stem (Gastal and Lemaire, 2002). Pask *et al*, (2012) published a detailed paper outlining the process in which winter wheat accumulates and uses nitrogen. In it, they discuss the three pools of nitrogen that are present in the plant; these include 'photosynthetic nitrogen' (PN), which consists primarily of Rubisco, 'structural nitrogen' (SN) which provides support to tissues and vascular connections of the shoot system (Lemaire and Gastal, 1997). Any further nitrogen that is not designated to either of these N pools is referred to as 'reserve N' (RN). Additionally, reserve N can be further classified into two types; Storage RN – which has a functional role of maintaining photosynthesis and grain nitrogen formation

during grain filling through remobilisation, and Accumulation RN – which arises from ‘luxury uptake’ of nitrogen and has no use throughout the grain filling period (Millard, 1988). These N pools are found in varying quantities within the plant fractions. The largest volume of SN was found in the true stem (evidently due to the structural nature/role of the stem), with accordingly less in the ear, leaf lamina and leaf sheath. Most PN was located in the leaf lamina and leaf sheath (clearly due to their photosynthetic nature), and again, with much less in the ear or true stem. Finally, the biggest quantity of RN was found in the true stem, with a modest amount in the ear, but less in the leaf lamina and leaf sheath. Furthermore, the greatest amount of nitrogen was remobilised from the leaf lamina, followed by the true stem, leaf sheath and finally the chaff. This shows similar results to experiments by Egle *et al. (*2008) on barley, where more N was taken from leaves than the stem during remobilisation. Pask *et al. (*2012) also went on to state, that the primary source of nitrogen (immediately post-anthesis) was the RN pool, as the green area remained mainly unaffected throughout the first half of grain fill, even though remobilisation had taken place. The largest N remobilisation was from the true stem, indicating that RN acts to ‘buffer’ the remobilisation of PN from the leaf lamina and leaf sheath to prolong the green area and thus maximise Radiation Use Efficiency (RUE). Senescence finally occurred mostly in the second half of the grain filling period and was related to mobilisation of PN.

Feller and Fischer (1994) concluded that senescence is the final stage of leaf development and is defined by the shift from nutrient assimilation to nutrient remobilisation. The rate of senescence and the remobilisation of leaf nitrogen are related to the nitrogen nutrition status of crop and on the source-sink ratio (Masclaux *et al*., 2000). The main resource of nitrogen for the developing grains of cereals is the nitrogen that is remobilised from the vegetative mass of the plant (Simpson *et al*., 1983). The amount of nitrogen that is remobilised is governed not only by the nitrogen remobilisation efficiency, but also the amount of nitrogen that is accumulated in the vegetative mass of the plant. The amount of nitrogen that is amassed in the crop at anthesis can estimate the amount of nitrogen available to be remobilised (Cox *et al*., 1986) while the quantity of nitrogen that accumulates in the

vegetative mass is governed by genotype (Clark, 1983), nitrogen fertiliser rates (Anjana and Iqbal, 2007), soil nitrogen availability (Xu *et al*., 2012) and the conditions throughout the growing season (Papakosta and Gagianas, 1991). Mickelson *et al. (*2003) found a strong positive correlation between N remobilisation and total yield as well as nitrogen yield. The vast majority of nitrogen located in cereal grains at harvest is derived from nitrogen that is remobilised from vegetative plant parts. In cereals, it has been demonstrated that between 50- 90% (Heitholt *et al*., 1990; Spiertz and Ellen, 1978) and even up to 98% in one experiment (Suprayogi *et al*., 2011) of grain nitrogen is remobilised from vegetative organs while the remainder is accounted for by post-anthesis uptake. The photosynthetic proteins (including Rubisco) are degraded promptly during leaf senescence (Feller and Fischer, 1994) which results in a decrease in photosynthetic capacity.

2.3.6 **Senescence of leaves and N remobilisation in the plant**

The large quantities required by the grain during the reproductive period of growth cannot be satisfied by supplies from the root; the additional required nitrogen is remobilised from the vegetative parts of the plants, especially the leaves (Spiertz and Devos, 1983). The level of N taken up throughout the vegetative growth phase in barley can range from 10-100% of total plant uptake. This is then mobilised and translocated during the period of grain filling. (Carreck and Christian, 1991; Bulman and Smith, 1994). Studies have shown that for cereals, oilseed rape and legume crops, commencement of the grain fill period is crucial as N uptake and N_2 fixation declined after flowering/anthesis and throughout seed maturation (Salon *et al*, 2001) and for some plants may even cease completely after anthesis. As already mentioned, the contribution of N remobilised from the vegetative mass to the ear in small grained cereal crops such as barley, wheat and rice can account for 90% of grain N (Gregersen *et al*, 2008; Kichey *et al*, 2007). For barley, it appears that this value fluctuates in relation to pre-anthesis N uptake. The level of fertilisation and source/sink relationships of a crop can influence the rate and timing of senescence and remobilisation of leaf nitrogen within the plant (Ono *et al*., 1999; Masclaux *et al*., 2000). There are however, conflicting results regarding this theory. Egle *et al*,

(2008) found that plants of optimum N status began to remobilise N at an earlier stage than low N fed plants (because low N fed plants continued taking N from the growth substrate (soil)). They also showed that such optimum N fed plants remobilised a greater proportion N from their leaves than low N fed plants. This disagrees with the results of Ta and Weiland (1992) that showed N remobilisation starting earlier when a plant receives a low N fertiliser level compared to a high N fertiliser level. Nitrogen remobilisation is activated not just by the process of grain filling, but also by each potential organ (e.g. leaf) that emerges. These new sinks can stimulate N remobilisation from existing/older plant parts (Masclaux-Daubresse *et al,* 2008). These emerging organs create N fluxes within the plant throughout the growth cycle, such as: leaf to leaf in the vegetative phase of growth (Wendler *et al,* 1995) and leaf to grain in the reproductive phase (Masclaux-Daubresse *et al,* 2008). It has been shown that delaying leaf senescence also delays nitrogen remobilisation (Diaz *et al*, 2008). This consequently results in a negative correlation between grain nitrogen concentrations and yield (Beninati and Busch, 1992). This presents the predicament that breeding plants with delayed leaf senescence would ultimately produce higher yields but lower grain nitrogen concentrations (Masclaux-Daubresse and Chardon, 2011).

2.3.7 **How N is remobilised**

Mesophyll cells lose their photosynthetic capacity early on during leaf senescence, while nitrogen metabolism converts from assimilation to remobilisation (Feller and Fischer, 1994); also, the roots ability to continue nitrogen uptake is indirectly impacted (Bertheloot *et al*., 2008). Leaf senescence is not a passive and unregulated process. During senescence, leaf cells undergo an orderly sequence of changes within the cell structure, metabolism, and gene expression. The earliest and most significant change in cell structure is the breakdown of the chloroplast (that contains up to 70% of the leaf protein). Metabolically, carbon assimilation is replaced by catabolism of chlorophyll and macromolecules such as proteins, membrane lipids, and RNA. Increased catabolic activity is responsible for converting the cellular materials accumulated during the growth phase of leaf into exportable nutrients that are

supplied to developing seeds or to other growing organs (Lim *et al*., 2007). During grain filling, the process of leaf senescence decreases green leaf area, while grain filling from current photosynthesis, nitrogen uptake by the roots and remobilisation from plant fractions occurs (Pommel *et al*., 2006). Carbohydrates and nitrogen amass to significant quantities in the vegetative mass of cereals prior to anthesis (Papakosta and Gagianas, 1991). The authors also raised the question as to what extent such a reserve contributes to grain yield and grain nitrogen yield. Papakosta and Gagianas (1991) found that the larger the quantity of dry matter or carbohydrates (and nitrogen) accumulated in the plant vegetative mass, the higher the translocation rates of dry matter to the grain, in agreement with other studies from Palta and Fillery (1995b); although these are extrapolated from Mediterranean conditions where post-anthesis stress such as heat and drought are common while in cooler, more temperate climates, post-anthesis assimilation would be greatly influenced. Furthermore, they also noted that grain yield was positively correlated to dry matter accumulation at anthesis. Such pre-anthesis reserves possess the potential to buffer grain yield against unfavourable conditions for photosynthesis during the grain filling period (Austin *et al*., 1980). The contribution of pre-anthesis assimilated dry matter to the grain yield of barley was estimated to contribute up to 74%, and up to 57% of grain yield in wheat (Gallagher *et al*., 1975b; Saeidi *et al*., 2012 and Gallagher *et al*., 1976 respectively). Differences in the efficiency of translocation during the grain filling period were attributed to retaining a quantity of dry matter at anthesis to maintain survival and biological functions, with the balance available for translocation (Papakosta and Gagianas, 1991).

2.3.8 **Carbohydrate Accumulation**

The rate of grain filling is reliant on the availability of current photoassimilate supply and the capacity for remobilising carbohydrate reserves from vegetative mass or organs to the developing grains (Li *et al*., 2013) and is considered a substantial compensation mechanism for maintenance of stable grain filling during periods when current photosynthate supply is limited due to abiotic stresses (Ehdaie *et al*., 2006). Austin *et al. (*1980) found that approximately half of the aboveground dry matter of

modern wheat and barley cultivars is located in the grains and that the total dry weight of the non-grain parts is usually less than the weight at anthesis. It was concluded by Schnyder (1993) that such relationships indicate that most of the photosynthate produced during the post-anthesis period and probably some photosynthate produced before anthesis is used for grain filling. The assimilation of carbohydrate pre-anthesis can contribute to final grain yield in cereal crops. Research carried out by Austin, *et al. (*1980) found that pre anthesis assimilation contributed up to 44 per cent of grain dry matter, in 1976, which was a very dry and hot year. This compares to only 11 per cent in 1977, a wetter and cooler year. This demonstrates the importance of pre-anthesis assimilation towards final grain yield; evidently illustrating the contributions made in contrasting environments, which agrees with Przulj and Momčilovič (2003) and Egle *et al. (*2008). Post-anthesis assimilation as a source of matter for grain filling depends on viable light intercepting green surfaces. These diminish due to natural senescence and the effect of different stresses, and depletion of carbon and nitrogen use for grain filling (Austin *et al*., 1980: Gaunt and Wright, 1992).

Many experiments have been conducted that manipulated photosynthesis (source) and grain number (sink) to answer the question if grain filling is limited by the photosynthetic activity of the plant or by the uptake capacity of the grains. Where photosynthesis was decreased, the decrease was not proportional to the decrease in the rate of grain filling or had no effect on grain filling; whereas in experiments that removed grains, there was generally not a significant response to the treatment (Jenner and Rathjen, 1975; Rawson *et al*., 1976; Martinez-Carrasco and Thorne, 1979). These results suggest that, although most of the photosynthate produced post anthesis is used for grain filling, photosynthesis and the concurrent rate of photosynthate deposition are not closely linked, and that the observed independence (is partly) in the operation of temporary storage pools. These temporary storage pools not only facilitate the storage of photosynthate during periods of excess production and provision of photosynthate to the grains during periods of low photosynthate production (Schnyder, 1993), but also operate throughout the diurnal cycle, as the carbon balance of plants is negative during the dark periods of the day, but photosynthate translocation is constant throughout the day (Jenner and Rathjen,

1972). These reserve pools also supply assimilate during the latter part of grain filling when photosynthetic capacity is decreasing due to senescence and the rate of dry matter accumulation of the grains exceeds the rate of dry matter accumulation in the total crop. These reserves maintain carbon supply when current photosynthate is insufficient to meet the needs of the grains and of other sinks. Therefore, there may be the necessity of a low-capacity fast response storage system as well as a high-capacity long-term storage system.

2.4 Prediction Methods

2.4.1 Introduction - Benefits of predicting grain protein

The grain nitrogen concentration of malting barley is a major quality criterion. Maltsters have requirements for nitrogen concentrations of 1.52-1.84%. Nitrogen concentrations below the minimum value result in insufficient nitrogen for enzyme activity, whereas, nitrogen concentrations above this range result in less alcohol and excess nitrogen produces a cloudy beer (Petterson and Eckersten, 2007). Essentially, producer profitability is governed by the balance of production costs, yield response and crop value. There is a financial incentive to produce wheat with high nitrogen concentration for bread milling, while there is also a financial incentive to produce barley for malting markets, with not low, but limited or restricted nitrogen concentration lower than 1.84% (Hansen *et al*., 2002; Bertholdsson, 1999).

The ability to provide advanced site specific predictions of grain N concentrations could offer many advantages to industry and producer alike. It may afford the producer the prospect of dividing a crop into various management zones to facilitate different practices to achieve the required produce qualities such as remedial action of variable rate fertiliser application or harvest planning to ensure delivery of produce within the accepted N concentration range. Such applications may present opportunities as a planning tool for the grain industry for purchasing and pricing of grain via regional predictions of N concentration and yield.

Grain N concentration is determined by both grain yield and grain N accumulation per unit area. The ability to accurately predict both of these parameters independently should facilitate the consistent and accurate prediction of grain N concentration. Analysing a number of crop and environmental parameters including soil nitrogen availability at ear emergence to establish the level of N available for post anthesis uptake, nitrogen content in the plant at ear emergence to determine the quantity of nitrogen available in the plant for nitrogen retranslocation to the developing grains, plus prediction of final grain yield should provide indications to the extent of the dilution effect occurring within the crop. The use of such a prediction method should meet a number of criteria. The technique should be simple, quick and ultimately

cheap for producers to utilise while simultaneously maximising accuracy of prediction.

2.4.2 Crop Accumulated Nitrogen

2.4.2.1 Destructive

One of the most traditional and reliable methods of calculating N accumulation in the crop is through sampling the crop, with subsequent destructive measurements using either the Kjeldahl method or the Dumas method. The Kjeldahl method of nitrogen determination is commonly considered as the standard or reference against which other processes of nitrogen analysing is ranked. The Kjeldahl method is a wet oxidation digestion that uses sulphuric acid (H_2SO_4), a variety of catalysts and salts to convert organically bound N in plant tissue to ammonium (NH_4) (Horneck and Miller, 1998). However, the Kjeldahl procedure does not recover 100% of the N in most samples, as nitrate (NO_3) and nitrite (NO_2) are not recovered; it requires a predigestion process which reduces nitrate to ammonium, but further, some nitrogenous compounds will not be converted due to a resistance of this process. (Simonne *et al*., 1994). The analysis of NH_4 can then be conducted by means of either an ammonium electrode, a continuous flow autoanalyser or steam distillation. Automated N analysers (based on the Dumas combustion method) which employ combustion for N determination have now replaced the Kjeldahl digestion method (Schmitter and Rihns, 1989). These dry combustion N analysers can recover significantly more nitrogen than the Kjeldahl as all the nitrogen in the sample can be recovered (Simonne *et al*., 1994). These combustion methods involve igniting the samples in an induction furnace at temperatures between 800-1,000°C in helium (He) and oxygen (O_2). An aliquot is passed through a catalyst to remove oxygen and convert nitrous oxides to N_2, eliminated of moisture and carbon dioxide. Nitrogen content is then determined by thermal conductivity (Muñoz-Huerta *et al*., 2013). The Dumas combustion method is now regarded as a standard process for nitrogen determination as it is less labour- and chemical- intensive and thus, more environmentally-friendly compared to the Kjeldahl method. However, the Dumas method does have shortcomings, including incomplete combustion causing loss of

nitrogen in the sample and the requirement of a small sample (Watson and Galliher, 2001). The main disadvantage to these methods of N determination is their invasive and destructive natures which prevents further analysis of the sample. They are also very time consuming and require specialised laboratories, which makes them very costly.

2.4.2.2 Non-destructive

Recent years have seen the design and development of in-field non-invasive or non-destructive methods of nitrogen determination. These methods operate under the principle of optical plant properties, all which are affected by plant factors including water content, leaf senescence, diseases, nutrients and plant nitrogen status (Zebarth *et al*., 2009). They operate on the fundamental transmittance properties of leaves, leaf chlorophyll fluorescence, canopy reflectance, satellite imagery and digital imagery processing. As a plant's optical properties change with nutritional variation, their electrical properties are also affected by physiological and nutritional status (Muñoz-Huerta *et al*., 2013). The benefit of these methods of N determination over that of the destructive methods include the obvious non-invasive characteristics which allow continuous monitoring and analysis.

Leaf chlorophyll meters measure the chlorophyll content of leaves which is a nitrogen status indicator due to it being an essential element of photosynthetic protein synthesis and is responsible for leaf colour (Demotes-Mainard *et al*., 2008). Their compactness and portability, quick response and affordability are positives, but there are nonetheless some negatives factors. Plant growth stage, cultivar variety, soil water and nutritional deficiencies other than nitrogen can affect chlorophyll measurements and therefore distort results. Also, leaf chlorophyll readings do not possess the sensitivity to distinguish between various nitrogen treatments (Perry and Davenport, 2007).

Another method of plant N determination are canopy data processing systems which are based on processing reflected electromagnetic energy. This method can estimate a number of plant biochemical and biophysical properties including leaf area index (LAI), aboveground biomass (AGB) and nitrogen concentration (Stroppiana *et al*.,

2009). Other indices have been calculated from combinations of canopy reflectance measurements including normalised difference vegetation index (NDVI), ratio vegetation index (RVI) and other indices. These are determined with the use of red, green and near-infrared reflectance data of a crop. The function of the light bands of satellite imagery relies on the basics that, for a healthy green leaf, there is a significant increase in the electromagnetic reflectance in the near-infrared region which is immediately next to the visible red band with high-energy absorption. This change from high-energy absorption (red band) to high-energy reflectance (near-infrared band) is the result of the plant's own natural protection mechanisms to prevent overheating, which is known to irreversibly denature the protein (Jensen, 2000). Additionally, a strong correlation between mid-infrared band and grain N concentration has also been established. This was due to the reflectance associated with the volume of water present in the leaves of the plant canopy (Jensen, 2000). The reflectance of the mid-infrared region increases simultaneously with decreases in plant moisture content.

Such sensors can be classified as either passive or active, depending on their light source, with passive crop canopy reflectance sensors utilising crop canopy reflectance provided by sunlight, such as the hand-held FieldSpec spectro-radiometer sensor (Analytical Spectral Devices, Inc., Boulder, CO, USA) or the CropScan, a multispectral radiometer (CropScan, Inc., Viola Heights Lane, NE, USA) while active sensors possess their own light source and therefore are not dependent on sunlight, including the tractor mounted Yara N-Sensor (Yara International ASA, Oslo, Norway), the hand-held GreenSeeker (NTech Industries Inc., Ukiah, CA, USA) or the Crop Circle (Holland Scientific, Lincoln, NE, USA) (Muñoz-Huerta *et al*., 2013). Additionally, satellite images captured from space-mounted sensors can also provide information on nitrogen management on large scale applications (Li *et al*., 2010). There are a number of issues with such methods including high cost, weather conditions such as clouds causing interference, slow processing of images with possible delay in use of available information and therefore delayed application of agronomic practices (Li *et al*., 2010; Goffart *et al*., 2008).

Finally, digital imaging methods for N estimation can calculate stress and biophysical plant parameters, such as tiller densities across a field, insect damage and nutrient and water deficiencies (Graeff *et al*., 2008). One of the main advantages of this method compared to the aforementioned is it does not require sophisticated instruments, but a commercial digital camera and an image processing system.

2.4.3 **Soil Nitrogen**

Soil mineral nitrogen (SMN) which varies with temperature (Leirós *et al*., 1999) and moisture (Sierra, 2002; Cassman and Munns, 1980) is frequently used as a factor in methods of recommending nitrogen fertiliser application rates for crops, based on crop yield potential, in conjunction with potential rooting depth. It is scientifically based and provides accurate results (Houlès, 2004). Soil mineral nitrogen tests can facilitate the reduction in nitrogen rate applications and therefore provide reductions in production costs for the producer while simultaneously benefiting the environment through reduced loss to groundwater by minimising the residual nitrogen in the soil at harvest (Bundy and Andraski, 2004). The timing of sampling of soil nitrogen supply will depend on the fate of the information to be utilised; although winter sampling will suggest soil nitrogen immediately available to a crop it is acknowledged that spring sampling may be superior as it eliminates the uncertainty associated with losses which occur over winter (Knight, 2006). This also agrees with the theory that the highest accumulation of soil mineral nitrogen generally occurs in early spring (Jungk and Wehrmann, 1978). Although research has shown that SMN is located throughout the soil profile from 0-90cm and deeper, half of the total is located in the 0-30cm region while the remainder is located in the 30-60cm and 60-90cm regions of 30% and 20% respectively (Chaney, 1990). Furthermore, research by Harrison (1995) has shown that SMN at the 60-90cm depth is closely correlated to that in the 30-60cm region, and sampling further than 60cm does not affect nitrogen recommendation rates.

Although soil mineral nitrogen testing will determine that which is immediately available, the total reserve of organic nitrogen in the soil, that which originates either

from crop debris or indigenous organic matter and the ensuing mineralisation can provide significant contributions towards mineral nitrogen available for plant uptake. The level of soil mineral nitrogen present in long term arable cropped fields has been estimated to be less than 1% of total nitrogen (Chambers *et al*., 1991). There are a number of methods which have been used to predict the mineralisation of nitrogen or the amount of nitrogen that will become available during the season (potentially available nitrogen – PAN) such as measurement of topsoil organic matter content, anaerobic incubation of soil samples or computer modelling (Knight, 2006) but no one method consistently provides superior prediction results above others (Benbi and Richter, 2002).

At the moment, there is no satisfactory Irish laboratory test for N in soils at farm level. In Ireland, nitrogen fertilisation rates are limited by the European Nitrates Directive (91/676/EEC). The permitted level of nitrogen that can be applied to agricultural crops is governed by the soil nitrogen (N) index system, which indicates the soil's ability to supply N during the growing season and depends on the previous cropping history and previous organic manure applications. It consists of four N indices ranging from 1-4, with the lowest soil N reserves in Index 1, with soil nitrogen reserves subsequently rising to the greatest quantities in Index 4. It is also divided into two classifications, those crops grown in land that is in tillage more than 5 years (and therefore away from long term grass ley) and those crops cultivated in soil less than five years from a long term grass ley (Wall and Plunkett, 2016).

If pre-planting soil mineral nitrogen tests are carried out (to estimate fertiliser nitrogen rates) in conjunction with in-season soil mineral nitrogen tests (ear emergence), it would be possible to conclude not only the level of nitrogen removed from the soil either via plant uptake and or losses but additionally, should give an indication of soil N availability for post anthesis uptake. According to the Nitrates Directive, pre-planting soil nitrogen supply is not known, and is estimated by means of previous crops and application of organic manures, while in-season soil tests at anthesis would provide indications of soil nitrogen available for uptake post anthesis. This review has not revealed any published literature that incorporates soil mineral nitrogen in prediction methods of grain nitrogen concentration.

2.4.4 **Yield Prediction**

As discussed previously, the price premium paid to producers of malting barley is reliant upon achieving a grain nitrogen concentration within the acceptable range set by industry; furthermore, producers strive to maximise yield to increase profitability per unit of ground cultivated. Hay and Walker (1989) stated that the yield of a crop is determined by three major processes, firstly, the interception of incident solar irradiance by the canopy; secondly the conversion of the intercepted radiant energy to potential chemical energy and finally, the harvest index. The interception of incident solar radiation is governed by the photosynthetic area of the canopy, whereas the conversion to potential chemical energy is dependent on the overall photosynthetic efficiency of the crop. However, an inherent inverse relationship exists between grain nitrogen concentration and grain yield (Simmonds, 1995; Kibite and Evans, 1984). Acreche and Slafer (2009) suggested that the cause of the negative correlation that exits between grain yield and grain nitrogen concentration is the result of the dilution effect. To overcome this inverse relationship it is possible to adjust agronomic practices. Fertiliser management that delays application until heading can increase grain N concentration, without reduction in yield (Bogard *et al*., 2010). The final grain yield of small grain cereal crops such as wheat and barley is determined by the total biomass production of the crop and the fraction of biomass allocated to the grains, known as the harvest index (Van den Boogaard *et al*., 1996). The relationship between grain yield and biomass at anthesis and/or during grain filling has been displayed in barley by Ramos *et al*. (1985). There are three traditional methods of assessing plant biomass, which include in-situ destructive, in-situ non-destructive and remote sensing.

2.4.4.1 Destructive

The most accurate method of assessing biomass is destructive sampling involving harvesting of the sample (at the desired growth stage), counting the number of plants in the sample area and recording the weight post oven drying. The biomass of the crop can then be presented on a dry weight basis either as dry weight per plant or as dry weight per unit area (Villegas *et al*., 2001). The leaf area index (LAI) can express

changes in the crop canopy through leaf area expansion. It is the ratio of leaf area to the area of the ground upon which the crop is occupying and is determined by establishing the average one-sided leaf area and relating that to the area of ground the crop is growing on or for the sample area, usually expressed in square metres. The green area index (GAI) demonstrates changes in total green area of the plant, (leaf, stems, ears, etc) to the ground it is growing on and again, it can be expressed on either a per plant or per unit area basis (Royo *et al*., 2004). Such methods of destructive sampling are invasive, time consuming and labour intensive but are additionally prone to sampling errors (Whan *et al*., 1991).

2.4.4.2 Non-destructive

Plant development, stress and yield capacities are exhibited in spectral reflectance from crop canopies and can be calculated using spectral vegetation indices (Weigand and Richardson, 1990). The measurement of spectra reflected by crop canopies at different wavelengths through photosynthetically active radiation (PAR) and near-infrared radiation (NIR) regions of the electromagnetic spectrum can estimate simultaneously, rapid and non-destructively, the photosynthetic traits such as green area and radiation use efficiency (RUE), which are vital components in determining yield (Peñuelas, 1998).

Tucker (1979) described vegetation indices (VI), taking the Normalised Difference Vegetation Index (NDVI) as an example, which is classically produced as a sum, difference or ratio of two or more spectral wavelengths. These NDVI measurements are highly correlated with photosynthetic activity in non-wilted plant mass and provide good predictors of plant canopy biomass, vigour or stress. When consecutive vegetation indices are recorded regularly over the course of the growing season of the crop, a seasonal profile can be developed graphing the progression of the crop canopy emergence, maturation and senescence that reveals crop performance and ultimately, crop yields (Boissard *et al*., 1993). The most commonly utilised of these indices are for the assessment of the characteristics associated with total photosynthetic area of the crop canopy, with the simple ratio (SR) and NDVI spectral vegetation indexes being the most frequently employed (Aparicio *et al*., 2000). The

ratio between the reflectances in the near-infrared (NIR) and red (RED) wavelengths is high for dense green vegetation but low for the soil, therefore giving a contrast between the two surfaces (Peñuelas *et al*., 1997). These indices have been strongly correlated with the absorbed photosynthetic active radiation (PAR), the photosynthetic capacity of the canopy and net primary productivity (Sellers, 1987). Estimates of biomass used to estimate grain yield, that are taken early in crop development can change rapidly. Additionally, as Quarmby *et al. (*1993) showed, yields begin to stabilise 50-100 days before harvest, indicating that yield estimates could possibly be calculated two months before harvest. However, these findings may not be applicable where conditions during grain fill are not limiting due to heat or water stress.

An additional non-destructive method of biomass measurements has been developed using digital image analysing; along with estimating biomass, it also has the capacity to express biomass distribution but additionally, when conducted repeatedly, can demonstrate growth rates. This method entails capturing digital colour images of the silhouette of the plant on a dark background. The image is then processed using image analysis software. This system predicts the biomass of plant individuals from the projected area of their silhouette on digital images. This method works on the assumption that the individuals are radially symmetrical with the erect stem being the axis of symmetry, which should be a linear function of the original surface area of the plants. Furthermore, the biomass of the plants is linearly related to their volume if the tissue density is constant. Therefore, biomass can be calculated from the two dimensional surface by a power function, due to a simple allometric relationship (Tackenberg, 2007).

Finally, one of the simplest methods of predicting grain yield of cereal crops is the yield components calculation. This method is based on the fundamental principle of crop components of yield parameters which make up final grain yield. Essentially, the numbers of grains m^{-2} are counted (i.e. grains/ear and ears per m^{-2}) and multiplied by the average individual grain weight (Slafer, 2007). As average individual grain weight data would not be available at ear emergence, when yield prediction would be carried out, it may be possible to substitute actual grain weight with a cultivar

specific grain weight average. This method may provide adequate accuracy of yield prediction within a satisfactory prediction target range, as research has found that individual grain weight does not influence grain yield to the same extent that grain number does (HGCA, 2003). An important factor to remember when estimating crop yield is the difference between grain yield and yield potential. Yield potential is the yield of the crop under the ideal growth conditions while grain yield is that which is harvested. Bearing this in mind, when such predictions are made (i.e. around anthesis) it is assumed that conditions preferential to achieving maximum yield potential will occur during grain filling. In the scenario where conditions do not facilitate such growth, yield estimates will be overestimated. Non-destructive methods of yield and biomass prediction offer rapid results and the potential of large-scale field evaluations. However, these methods can be expensive in terms of time and financial resources and partly require special equipment. On the other hand, the simple method discussed which utilises the components of yield is not costly, but is labour intensive, reducing the opportunity of large-scale evaluation on broad-acre crops.

2.5 Evaluated Experiments of Prediction Methods

Considering the parameters which this review believes may be utilised to predict grain N concentration at harvest, an appraisal of a selected number of experiments carried out by researchers in varying environments is included. The methods which were employed in each experiment are included and an evaluation of the capacity to be employed or developed to aid the prediction of grain N concentration in Irish conditions is provided.

Molina-Cano *et al. (*2001) conducted research examining the capacity to predict final grain nitrogen concentration of malting barley. Working on the basis that that nitrogen accumulated in the vegetative mass of a plant at anthesis is positively correlated to final grain nitrogen concentration (Austin *et al.,* 1977; Garcia Del Moral *et al.,* 1985) since the main source of the grain nitrogen in cereals is the nitrogen in leaves (Jenner *et al.,* 1991). Molina-Cano *et al. (*2001) found that by

using linear regression equations it was possible to predict the nitrogen concentration of grain by analysing the nitrogen content of the whole plant harvested at anthesis. This experiment was conducted by the authors in the hot, dry regions of Mediterranean Southern Spain. Grain filling of crops in such environments, which are often subjected to heat and drought stresses rely on pre-anthesis reserves of carbohydrates and nitrogen to contribute to the starch and nitrogen content of the grain (McCraig and Clarke, 1982). This dependence on pre-anthesis reserves can be attributed to the reduced nutrient uptake from the soil due to the effect of water stress (Nahr and Gretzmacher, 2002). Nitrogen and carbohydrate in the crop will have reached a maximum at anthesis, so, it is solely the contribution of this accumulation that can impact grain nitrogen concentration. In growing environments where water stress is not an issue, post anthesis nutrient uptake can provide significant contributions, influencing grain nitrogen concentrations.

Hansen *et al. (*2002) used a combination of repeated canopy reflectance measurements taken at a number of growth stages and partial least square regression in an attempt to predict both grain yield and nitrogen concentration in winter wheat and spring barley. In this experiment predictions of dependent variables were related to repeated canopy reflectance. These prediction method systems differed by the volume of data which they employed; one system (PLS1) used one data measurement from the last date while the other system (unfold-PLS1), used all the data gained from the measurements in expectation of predicting yield and grain protein. It was revealed that the prediction system which used just one (the final) sampling date provided as equally good) a prediction of yield as the system which employed data from all sampling dates (correlation between measured and predicted yields were greater than 0.96 for both). These spectral measurements appear to be correlated to nitrogen application, and therefore provide a respectable prediction of grain yield but a poor estimate of grain nitrogen concentration (mostly in barley) as yield responds to nitrogen considerably, whereas nitrogen concentration is related to events, such as nitrogen mobilisation, which occurred after measurement. As both prediction methods produced comparative results, the system which uses the least number of data or parameter inputs should be used for simplicity of use and to maintain the models robustness against over-fitting of parameters.

Pettersson (2007), utilised observations of soil conditions, sowing day, fertiliser rate, remote sensing at early stem elongation and the temperature sum during grain filling in a prediction model, to predict the grain nitrogen concentration of malting barley. It was concluded that it was possible to make predictions (for specific cultivars) of malting barley grain N concentrations at harvest using day number from sowing and vegetation index at GS 32 (when it would be still possible to adjust fertiliser management) (R^2=0.83). However as suggested by the authors, the effectiveness of the sowing day parameter may be due to the thermal stress during grain filling; as sowing date is delayed, grain filling often occurs simultaneously during higher temperatures. This would also provide explanation to the reason why including the accumulated temperature during grain filling did not improve the model's prediction capacity.

Söderström *et al. (*2010) examined the capacity to predict grain N concentration of malting barley by using a Yara N-Sensor and satellite imagery in conjunction with regional data including weather data, quality analyses of the malting barley, crops grown and field boundaries in partial least squares (PLS) models. Although both models relied upon canopy reflectance, when weather data was incorporated it improved the accuracy. It was discovered that the N-Sensor provided more precise results than that of the satellite imagery. Söderström *et al. (*2010) utilised the findings of Börjesson and Söderström (2003), which conveyed that canopy reflectance in cereals at the end of anthesis (GS69) could be valuable in terms of calculating nitrogen nutrition status of a crop which corresponded to grain crude nitrogen concentration at harvest. Although both methods predicted the average grain nitrogen concentration value for the field of 1.74% (within the accepted N concentration range), the amount of variation associated with the satellite based prediction model was much larger than that of the Yara N-Sensor (SD 0.71% compared to 0.29% in the N-Sensor). The Yara N-Sensor was used at the end of anthesis (GS69), and in conjunction with weather data in partial least square models, provided reasonable prediction of grain N concentration at one site, for one year. When the data from both regions and years were included in the model, the prediction accuracy was not as strong, which was possibly due to the contrasting weather conditions present in both the growing seasons. The accuracy of the satellite prediction model was not as

successful as that of the proximal sensor (Yara N-Sensor) but when satellite data was examined against crop measurements from the ground, it provided valuable information for predicting the grain nitrogen concentration. One of the main constraints regarding the satellite is the weather condition or status at time of acquiring the image. Interference of image acquisition due to weather conditions such as cloud cover will impede results and consequentially, may affect timeliness of remedial action such as altering agronomic practices.

Hector *et al. (*1996) based their experiments on those conducted by Goyne, *et al. (*1994) and Goyne *et al. (*1996) to develop a model for predicting barley grain N concentration. The original model involved the interaction of five parameters, including phenology (temperature and photoperiod dependent), soil water balance (exponential decay in soil water content in each layer), leaf area index (logistic functions of thermal time, physiological and light competition senescence of leaves), dry matter accumulation (radiation interception and radiation use efficiency) and grain yield. The grain yield was estimated by using an assumed harvest index value; a maximum value of 0.45 was given when nitrogen was limiting, but this figure was successively reduced as nitrogen availability increased (sourced from experimental findings). Additionally, the model was altered to model the nitrogen balance and the effect of nitrogen deficiency on crop growth by including soil N, N uptake, N distribution within the plant and remobilisation. These were adapted from the methods of Sinclair and Amir (1992) that examined the nitrogen limitations on the growth and yield of spring wheat. As the previously mentioned authors worked on spring wheat, several alterations were made to the model to apply it to spring barley. Ultimately, Hector *et al. (*1996) found that it is possible to accurately predict grain nitrogen concentration under various soil nitrogen and water scenarios (R2=0.67%). They also stressed the importance of initial soil mineral N levels plus the timing and extent of water stress upon final grain nitrogen concentration, and reiterated that response of grain N concentration to nitrogen and water is site and season specific. It was concluded that the effect of nitrogen application rate on the probability of attaining grain nitrogen concentration levels within the quality limits, as well as maximising yield, can be calculated using such models with historical rainfall data for individual locations in key production regions. Hector *et al. (*1996) used this

model to predict grain nitrogen concentration in south-eastern Queensland, Australia, where the dry and hot climate would (similarly to that of Molina-Cano *et al*., 2001) reduce plant N uptake post anthesis. Although the model presented by Hector *et al. (*1996) considers initial soil mineral nitrogen, it does not facilitate nitrogen mineralised from the soil for plant uptake post anthesis due to heat and water stress.

As has been previously discussed, leaf nitrogen content at anthesis is positively correlated to grain nitrogen concentration. Zhao *et al. (*2005) carried out reflectance measurements to quantify leaf nitrogen concentration; furthermore, the results conformed to previous findings that leaf nitrogen concentration at anthesis is strongly correlated to grain N concentration (R^2=0.73; Wang *et al*., 2003). This reinforces the hypothesis that grain N concentration may be predicted from plant nitrogen concentration at anthesis. The authors also produced a vegetation index (VI) from the canopy reflectance measurements which correlated significantly (R^2=0.46) to leaf nitrogen concentration. Additionally, it was found that water stress can increase grain nitrogen concentration at the grain filling stage. It was discovered that leaf water content could be used to further predict grain nitrogen concentration at harvest through monitoring during the grain filling stage. The ability to predict grain N concentration via monitoring water stress or its increase in accuracy of predicting grain N concentration by its inclusion in a prediction system may offer potential to regions where heat and water stress commonly occur during grain filling. In other more temperate climates, such as that of Ireland, such a parameter is not beneficial towards predicting grain N concentration, except possibly in years of extreme weather fluctuations.

The methods developed and utilised by the authors and collaborators of the examined research papers predicted grain nitrogen concentration of wheat and barley to varying degrees of success. The aim of this project is to produce a prediction system that encompasses a number of criteria such as simplicity, ease of use, timeliness and affordability for the producer, while delivering a consistent and reliable accuracy of prediction.

Table 2.1. Literature summary of prediction methods

Author	Grain N Concentration Prediction Method	General Comments
Molino-Cano *et al*. (2001)	Whole plant N concentration at anthesis	Post-anthesis contributes little to grain N concentration due to heat and drought stress during grain filling
Hansen *et al*. (2002)	Repeated canopy reflectance and partial least square means	Provides a good prediction of yield, but poor estimate of grain N concentration
Pettersson (2007)	Observations of soil, sowing day, fertiliser rate, remote sensing at early stem elongation and the temperature sum during grain filling	Temperature sum improved prediction as grain filling regularly occurs during higher temperatures
Söderström *et al*. (2010)	Yara N-Sensor and satellite imagery along with regional weather data, barley quality analysis, crops grown and field boundaries in partial least squares regression	Satellite imagery produced poorer results, which may have been due to weather conditions at time of analysis
Hector *et al*. (1996)	Adapted Goyne *et al*. (1994, 1996) using 5 parameters of phenology, soil water balance, leaf area index, dry matter accumulation and yield. Altered to include soil N, N uptake and N distribution and remobilisation	Does not account for N uptake during growing season - uptake may be reduced due to water stress and temperature
Zhao *et al*. (2005)	Canopy reflectance and leaf water content	Monitoring of leaf water stress would be less beneficial in temperate climates

3 Model Development Using Fertiliser N Experiments

3.1 Introduction

Grain nitrogen (N) concentration is a major quality criterion of malting barley, where a concentration of 1.52-1.84% is required for the brewing industry (Söderström *et al*., 2010). There can be a large degree of variation in grain N concentration due to both site (Stoddard and Marshall, 1990), seasonal (Triboï *et al*., 2000) and management factors such as fertiliser N supply (Lueck *et al*., 2006) and sowing date (McLeod, 1992). A system to predict grain N concentration (GNC) would be useful to both producer and industry alike. Prediction early in the season would allow application of remedial N fertiliser for crops identified as being at risk of having grain N concentration lower than that required (Vaughan *et al*., 1990; Stark and Tindall, 1992). Such a possibility would also afford the producer the ability to create management zones to prioritise harvest of those crops predicted to satisfy grain N market requirements (Hansen *et al*., 2002). Additionally, such a prediction system would aid industry in accurate estimation of harvest volume and subsequent price structuring.

In order to predict grain N concentration from measurements of crop growth before flowering, it is necessary to understand what the grain characteristics (sub-components) are that determine N concentration in the grain at harvest, the variation that occurs in each of these components in response to environment and crop management and the relationship between these sub-components and crop traits at or before anthesis. These relationships are illustrated in Figure 3.1. Grain N concentration is the quotient of grain N content (kg N ha^{-1}) and grain yield (kg DM ha^{-1}) per unit area. In turn grain N content of small grain cereals is the result of total aboveground plant nitrogen content at harvest (kg N ha^{-1}) and N harvest index. Nitrogen harvest index (NHI) is the partitioning ratio of N between the grain and the total aboveground plant tissue at maturity (Austin *et al*., 1977). There are two widely

used approaches to quantitatively explain variation in grain yield; analyses of (1) yield components and (2) biomass and harvest index (Gallagher *et al*., 1975 and Van den Boogaard *et al*., 1996 respectively). The yield component approach interprets yield as the product of grain number per unit area and individual grain weight. Although grain weight is an important component of grain yield, variation in yield of barley between sites and seasons is largely associated with variation in grain number (Bingham *et al*., 2007a & b; Kennedy *et al*., 2016), a yield component that is determined in the critical period prior to flowering in barley (Arisnabarreta and Miralles, 2008; Kennedy *et al*., 2016). Grain yield can also be described as the product of total aboveground crop biomass and harvest index. Harvest index (HI) expresses the grain yield of a crop as a fraction of total aboveground dry matter production (Van den Boogaard *et al*., 1996; Donald and Hamblin, 1976). Harvest biomass can be positively or negatively influenced by environmental variation (as described by Hay, 1995; and references within).

A negative relationship between grain N concentration and yield of cereals has been widely reported (Simmonds, 1995; Oury and Godin, 2007; Bogard *et al*., 2010). Kibite and Evans (1984) concluded that such a negative relationship between grain N concentration and yield was either due to a limited quantity of N deposited in a large number of grains or the result of a limited amount of N diluted by a larger mass of carbohydrates. Similar conclusions were presented by Acreche and Slafer (2009). Thus, prediction of grain N concentration by anthesis may be achieved through the prediction of grain N content and yield and there are several ways, involving different sub-components, through which this might be achieved (Figure 3.1). How successful a particular approach is in predicting grain N concentration across crops and seasons will depend on the relative sensitivity of each of the sub-components to climatic and crop management factors and how robust their relationship is with pre-anthesis crop traits.

Pre-anthesis N uptake has been shown to contribute up to 90% of the total N in cereals at harvest (Spiertz and Ellen, 1978; Heitholt *et al*., 1990), although in some environments, post-anthesis nitrogen uptake (PANU) may contribute significantly to total plant N content and is related to grain nitrogen content (Perez *et al*., 1983). PANU appears to be influenced by soil mineral N concentrations prior to grain filling

and can be increased by applications of fertiliser N around this time (Van Sanford and MacKown, 1986: de Ruiter and Brooking, 1994; Heitholt *et al*., 1990). Thus there is scope for using soil mineral N measurements at ear emergence to estimate PANU. In contrast to harvest N content, estimating NHI at ear emergence is more problematic, because N remobilisation and transfer to the grain occurs after flowering. NHI of spring barley has increased with breeding for yield and is greater in N-deficient than N-sufficient crops (Bingham *et al*., 2012).

There are a couple of potential approaches for estimating yield from measurements at ear emergence. It may be possible to estimate final grain number from assessments of spikelet numbers per ear and ears per unit ground area at ear emergence. Spikelet numbers will provide an index of the number of potential grain sites assuming that ear fertility is consistently high. Grain filling in barley is generally considered to be sink limited (limited by the capacity to store dry matter rather than amount of assimilate available) (Bingham *et al.*, 2007a; Kennedy *et al.*, 2016). Thus in the absence of serious post-anthesis water stress or disease that could reduce photosynthesis, grain weight and harvest index may be relatively stable across environments. In fact there is evidence that the storage capacity of grains (potential grain size) is determined by climatic conditions and crop growth shortly before flowering (Bingham *et al*., 2007b). Consequently, estimates of biomass or potential grain numbers at ear emergence may be enough to provide a sufficiently robust prediction of yield when the risk of drought and late season disease is low.

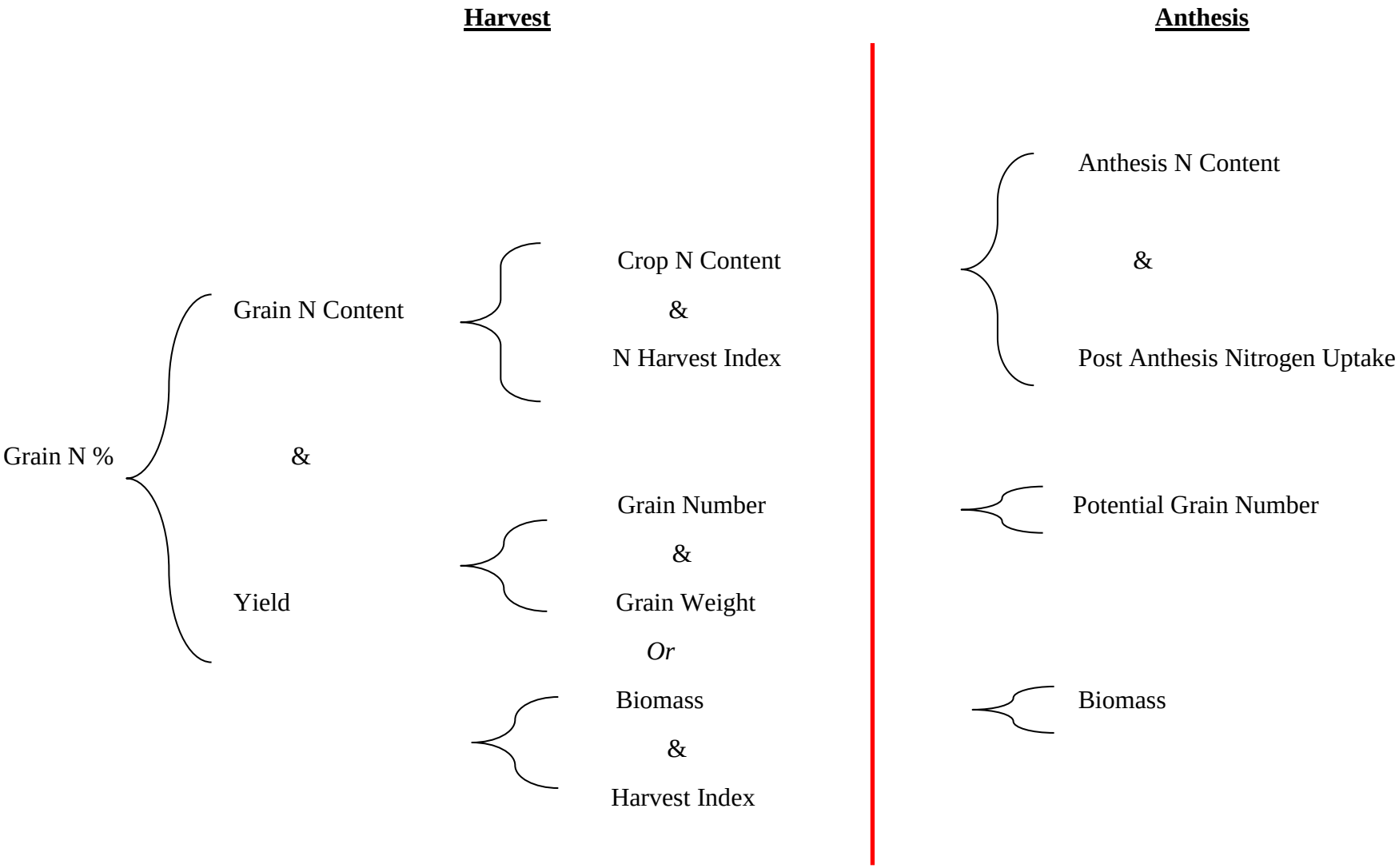

Figure 3.1 Flow diagram of sub-components influencing grain N concentration

A number of studies have tried to predict concentrations of N and protein in cereal grains. Grain N and protein concentrations are synonymous, since grain protein concentration is normally taken to indicate crude protein concentration, which is grain N concentration multiplied by a conversion factor, rather than true protein concentration (Magomya *et al*., 2014; Tkachuk, 1969).

Work carried out under Mediterranean conditions examined the potential to predict final grain nitrogen concentration of malting barley (Molina-Cano *et al*, 2001). Total plant nitrogen concentration at anthesis was related to grain nitrogen concentration. Linear regression analysis gave a highly significant correlation between both ($P<0.001$; $R^2 = 0.69$). The authors concluded that it is possible to predict grain N concentration from total plant N concentration at anthesis. However, a number of potentially important influencing factors were not examined in the model, for example heat stress during grain filling which is common in the Mediterranean environment. Research has shown that post anthesis N uptake is reduced by low soil water availability (Clarke *et al*., 1990; Gunes *et al*., 2006). Therefore, the main source of N for grain filling would be N remobilised and translocated from pre-anthesis stem and leaf reserves. Hence, the strong correlations found by the authors between total plant N concentration at anthesis when measurements were made and grain N concentration may not hold under conditions where post-anthesis N uptake is significant.

Australian studies examined the prospect of predicting grain N concentration in wheat and barley from available water and N at sowing (Dalal *et al*., 1997). While a system based on knowledge available at sowing may work in regions where growing season rainfall is low and predictable, and therefore soil water reserves at sowing have a large impact on yield, it is questionable if such a system would be reliable in areas with higher and less predictable rainfall. A concerning element of this prediction method of barley grain N concentration is it fails to take into account environmental changes that may occur from planting (when measurements and recommendations are made) to harvest. Significant changes to rainfall or temperature conditions would result in large under or over estimation of grain N concentration.

Zhao *et al. (*2005) examined the use of leaf N content and water stress measured at a number of sampling times including anthesis and grain filling to predict the grain N concentration of wheat across a range of varieties, fertilisation and irrigation treatments. Although there was a significant association between grain N concentration and the

explanatory variables the amount of variation accounted for was low ($R^2 = 0.36$). As the authors acknowledged, grain N concentration is determined by plant N accumulation at anthesis and the transfer efficiency to the grain during grain filling. The low amount of variance accounted for was attributed to the many factors that influence the N transfer efficiency from vegetative mass to the grain sites, including cultivar, irrigation levels and fertilisation rates. Water stress at the grain filling stage was also shown to have a significant influence on grain N concentration, and the authors therefore suggest that it is possible to forecast grain N content by monitoring plant water content during the grain filling stage of winter wheat. It was concluded that both leaf N content at anthesis and plant water stress at the grain filling stage can be used to predict grain N concentration. However, due to the low level of variation accounted for it would indicate that the prediction system lacks precision. Furthermore, measurements taken post-anthesis and during grain filling would reduce the available window for the application of N to rectify crops estimated to be at risk of a low grain N concentration.

Given the number of factors that can independently influence yield and grain N content, it is clear that prediction models based on single explanatory variables are unlikely to be satisfactory. More complex models that incorporate a larger number of variables are likely to be more robust, but may be more difficult to implement (Hansen *et al*., 2002). In addition, the larger the number of variables the greater the cost and effort required to measure them. Thus, for a prediction system to be useful in practice a compromise must be struck between the level of complexity required for acceptable precision and the ease with which users can measure the required parameters.

The aim of the research conducted in this chapter was to investigate the relationships between crop measurements made during the growing season and grain N concentration of barley at harvest. N-fertiliser supply was varied to generate the desired range of grain N concentrations of crops grown at different sites and in different years. By deconstructing grain N concentration into a hierarchy of sub-components (Figure 3.1) the specific objectives were to 1) identify which harvest sub-components relate most strongly to grain N concentration 2) to investigate the relationship between the most important components of grain N concentration at harvest and a range of anthesis crop traits 3) to use this understanding to develop statistical models to predict grain N concentration from selected anthesis crop measurements.

3.2 Materials and Methods

3.2.1 Site Description

Field experiments were conducted at three locations representative of the contrasting soil types employed in malting barley production systems in Ireland. The three sites were located at the Teagasc Crops Research Centre, Oak Park, and Carlow, Ireland and were located within 1 km of each other.

One experiment was conducted in 2013 located in Road Field (RF; 52.85^{o}N, 6.91^{o}W) and two in 2014; College Field (CF; 52.85^{o}N, 6.92^{o}W) and Bull Park (BP; 52.86^{o}N, 6.92^{o}W). All three sites are between 52 and 56m above sea level. The Road Field and College Field soil type, recognized as an Athy Complex (Conry and Ryan, 1968), is classified as a Grey Brown Podzol. It is a light textured, gravelly, coarse sandy loam, composed mainly of limestone with a small proportion of sandstone and granite. It is moderately deep (0.5-0.7m) and free draining, with 12-15% clay content in the plough layer. It has a good crumb structure and is easily tilled, although crops can be prone to drought particularly in dry seasons due to the low moisture holding capacity of the soil. The Road Field and College Field sites were in long term tillage with winter barley and spring barley the previous crops respectively. The soil in Bull Park belongs to the Mortarstown Series (Conry and Ryan, 1968b) and is derived from calcareous glacial till material composed mainly of limestone. The soil is a well-drained loam ranging between 0.5-0.75m with clay content of between 19-23% in the plough layer, while clay content increases up to 42 per cent in the B horizon. As a result the moisture holding capacities of these soils are high and roots can freely penetrate down to the parent material. The Bull Park site was in long term tillage with winter oats as the previous crop.

All three experimental sites were located within 1km of the Oak Park national meteorological station, from where meteorological data including solar radiation, rainfall and maximum and minimum temperatures were obtained.

3.2.2 **Treatments and experimental design**

The experimental design was a randomised complete block with four replications. The treatments consisted of a range of fertilizer N rates and timings. In 2013 these were applied to plots sown at a standard seed rate (300 seeds per m^{-2}) only. In 2014 the range of N treatments was altered compared to 2013 to create greater variation in crop canopy and grain N content and to attain an improved distribution of data points above and below the expected economic optimum N rate. Full details of treatments are given in Tables 3.1 and 3.2. Experimental plots were sown as two 2.3 x 9.3m plots with one plot designated for destructive sampling throughout the growing season and the other for non-destructive measurements and combine harvesting for determination of yield, grain N and other quality characteristics.

Table 3.1. Experimental treatments of Road Field, illustrating seed rate, total N rate, N timing and anthesis N application rates in year 2013.

Treatment	**Seed Rate (m^{-2})**	**Total N Rate (kg N ha^{-1})**	**N Timing & Rate (kg N ha^{-1})**		
			Sowing	**Mid-tillering**	**Anthesis**
1	300	0	0	0	0
2	300	90	30	60	0
3	300	120	30	90	0
4	300	150	30	120	0
5	300	180	30	150	0
6	300	210	30	180	0
7	300	30	0	0	30
8	300	120	30	60	30
9	300	150	30	90	30
10	300	180	30	120	30
11	300	210	30	150	30
12	300	240	30	180	30

Table 3.2. Experimental treatments of Bull Park and College Field illustrating seed rate, total N rate, N timing and anthesis N application rates in year 2014.

Treatment	Seed Rate (m^{-2})	Total N Rate (kg N ha^{-1})	N Timing & Rate (kg N ha^{-1})		
			Sowing	Mid-tillering	Anthesis
1	300	0	0	0	0
2	300	40	40	0	0
3	300	80	40	40	0
4	300	120	40	80	0
5	300	160	40	120	0
6	300	200	40	160	0
7	300	40	0	0	40
8	300	80	40	0	40
9	300	120	40	40	40
10	300	160	40	80	40
11	300	200	40	120	40
12	300	240	40	160	40
13	300	200	40	80	80
14	300	240	40	80	120
15	600	120	40	80	0
16	150	0	0	0	0
17	150	40	40	0	0
18	150	80	40	40	0
19	150	120	40	80	0
20	150	160	40	120	0
21	150	200	40	160	0

3.2.3 **Crop establishment and management**

Each experiment was sown with spring barley (*Hordeum vulgare* L.) using a Fiona tractor mounted seed drill with 11.7cm row spacing. Sowing dates were 26/03/2013 (Road Field), 10/04/2014 (College Field) and 22/04/14 (Bull Park). The two row spring malting barley cultivar variety SY Taberna was selected as it possessed a proven track record in the Department of Agriculture, Food and Marine (DAFM) Recommended Variety List.

Nitrogen treatments were applied as calcium ammonium nitrate (CAN 27%). Other than the N treatments, experimental plots were managed according to normal commercial practices. Details of cultivations, P, K and Mn fertilizer, and applications of herbicides, fungicides and insecticide at each site are given in Appendix 1.

3.2.4 **Crop measurements**

Destructive sampling was carried out at Zadoks growth stage (GS) 31 (first node detectable) (Zadoks *et al*., 1974), GS 39 (flag leaf fully visible), and GS 59 (ear completely emerged), and just prior to combine harvesting. On each sampling date plants were pulled by hand from four adjacent 0.5m row lengths at two random locations in each plot, to give a sampling area of 0.468 m^{-2}. These were bulked together as one composite sample and transported to the laboratory in sealed plastic bags where they were stored in the dark in a cold room at $4^{o}C$ to await processing. To avoid edge effects when sampling a minimum distance of 0.5m was maintained from the sampled quadrat area to the edges and ends of the plots. Furthermore, another gap of 0.5m was left between subsequent sampling areas. All processing was completed within 48 hours of sampling.

Protocol for plants sampled up to and including GS59 comprised cutting off and discarding of roots and weighing total sample fresh weight to the nearest 0.01g. The sample was then divided into ten equal subsamples by selecting shoots at random and placing them sequentially in consecutive bundles. The shoots in one randomly selected subsample were counted to determine total shoot number. Two subsamples were selected at random, bulked together, weighed fresh and separated into leaf laminae and stem plus leaf sheath, and at GS59 ear fractions; all plant fractions were then weighed

separately. Each plant fraction was then dried at 70^{o}C for a minimum of 48 h (or until constant weight) and dry weight measured. Post drying, stem/leaf sheath fraction of GS59 was separated into true stem and leaf sheaths, redried and reweighed. These plant fractions were used for determination of the partitioning of biomass and N in the different plant parts. A third subsample was weighed and separated into leaf, stem plus leaf sheath and ear. This subsample was used for green area index (GAI) determination. Projected area of leaf laminae, stem plus leaf sheath and ears were measured, separately, using a WD3 – WinDIAS Leaf Image Analysis System (Delta-T Devices Ltd, Cambridge, UK). The remaining seven subsamples were bulked together, weighed fresh and dried at 70^{o}C as described above and used for total biomass and crop N content determination.

At GS 59, ear measurements were also made. The number of spikelets (as a measure of potential grain sites) per ear and ear length were recorded for twenty randomly selected ears per sample.

The protocol for final pre-harvest samples comprised cutting off and discarding roots, weighing total fresh weight, selecting 20 shoots at random and weighing them. Selected shoots were separated into leaf, stem and associated leaf sheath, and ear and weighed. These plant fractions were then dried at 70^{o}C for a minimum of 48 h (or until constant weight) and dry weight measured. Post drying, stem/leaf sheath fractions were separated into true stem and leaf sheaths, dried at 70°C for 48 h (or until constant weight) and reweighed. These plant fractions were used for determination of biomass and N partitioning. Ears of the 20 randomly selected shoots were separated into grain and chaff by hand to calculate the number of grains per ear and additionally biomass partitioning and N content determination. The remaining pre-harvest biomass samples were threshed using a mechanical threshing machine (Wintersteiger AG, Austria) separating grain and straw/chaff and weighed, to determine both N and harvest index figures. Post threshing, straw and grain fractions were dried at 70°C for 48 h (or until constant weight) and reweighed. For all intact plant samples, the dried material was milled to <2mm in a cutting mill (RetschMühle. Retsch GmbH Haan, Germany). Plant fractions were finely ground (<1mm) in a hammer mill (Polymix – PX-MFC 90D, Kinematica AG, Switzerland). N concentration in milled samples was subsequently determined by means of the Dumas combustion method using a Rapid N Cube (Elementar Analysensysteme GmbH) using a sample size of approximately 400mg.

3.2.5 **Soil sampling and analysis**

Soil was sampled at GS59 and at harvest to measure available soil mineral N (nitrate and ammonium). Samples were collected with a 20mm diameter auger. Samples were collected from 0-30 and 30-60 at four locations in each plot and pooled together to give a composite sample for each soil layer. Samples were taken from selected treatments (the 0, 90, 120, 150, 180, 210 kg N ha^{-1} in 2013 and 0, 40, 80, 120, 160, 200 kg N ha^{-1} in 2014). Samples were maintained at 2-4°C in a cool box immediately after collection and subsequently stored at -20°C. For analysis of soil mineral N samples were allowed thaw for a period of 16 hours at 2-4°C in a refrigerator and extracted within 24 hours of start of the thaw. Freezing followed by slow thawing (16 h at 2-4°C) has been shown to alter the ratio of nitrate to ammonium in the extracts compared to those from fresh soil, but not the total mineral N content (Kindred *et al*., 2012).

For mineral N analysis, stones and plant residues were removed by hand and 100g of soil was shaken with 200ml of 2M KCl for 60 minutes on a rotational shaker (New Brunswick Scientific Model G-10 Gyrotary shaker). The soil slurries were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone UK) and the filtrate was frozen to await analysis at a later date. Ammonium and nitrate content was determined by colorimetric analysis using a Skalar San++ Automated Wet Chemistry Analyser also known as Continuous Flow Analyser (Skalar Analytical B.V, The Netherlands). Soil moisture content was measured gravimetrically by oven drying a subsample of the same soil at 105°C for 24 hours.

Dry bulk density of the soil was determined at each experimental site at two randomly selected plots per replication by taking soil cores of 10cm^3 from the mid-point of each upper horizon (0-30cm). The soil cores were dried at 105°C for 24 hours before weighing.

3.2.6 **Combine Harvesting**

Experiments were harvested on the 14/08/13 at the Road Field site and on the 13/08/14 and the 18/08/14 for the College Field and Bull Park sites respectively. Grain yield was determined by harvesting the designated 2.3 x 9.3m harvest plots using a Deutz-Fahr plot combine (Deutz AG). Grain from each plot was weighed by the harvester

independently and a sub-sample (approximately 2 kg) taken for moisture content and grain quality determination. Moisture content was determined using a DICKEY-John GAC 2000 (DICKEY-john Corporation**,** Auburn, IL, USA). Samples were subsequently oven dried before determination of thousand grain weight and protein concentration. Thousand grain weight (TGW) was calculated using a Pfeuffer – Contador grain counter while grain N was determined via Near Infra-Red (NIR) spectroscopy (Foss Infratec 1241, Foss A/S, Hillerød, Denmark). Grain N concentration was calculated from grain protein concentration using a conversion factor of 6.25.

3.2.7 **Calculations and statistical analysis**

Data for the standard seed rate treatments and fertiliser N rates without anthesis N applications (i.e. treatments 1 to 7 in 2013 and 1 to 6 in 2014) are analysed and presented in the current chapter. Data from the remaining treatments are analysed and presented in Chapter 4.

Calculations

Harvest index (HI) was calculated by expressing the dried grain weight of threshed samples as a proportion of the total dry matter of threshed samples:

$$HI = \frac{GY}{AGB} \; X \; 100$$

where GY is grain yield and AGB is the aboveground biomass.

Nitrogen harvest index (NHI) was calculated as given by Austin and Jones (1975):

$$NHI = \frac{GY \; x \; Nconc}{(straw \; DM \; x \; straw \; Nconc) + (GY \; x \; Nconc)}$$

where straw DM is the dry weight of the straw.

The number of spikelets per ear was multiplied by shoot number which was determined from quadrat samples to calculate potential grain numbers per m^{-2}. The length of ear was then correlated against spikelet number per ear to evaluate its ability to act as an indicator of spikelet numbers per ear and hence the potential number of grain sites per ear.

Nitrogen Nutrition Index (NNI) was calculated according to (Lemaire *et al*., 1989) as follows:

$$NNI = \frac{actual\ N\ \%}{critical\ N\ \%}$$

where critical N % is the N concentration (% DM) in the aboveground biomass at the optimum fertiliser N supply and actual N % is the N concentration (% DM) in the aboveground biomass for any given sample at the same sampling time. Values of NNI ≥ 1.0 indicate that N supply to the crop is optimum or in excess, while values of NNI <1.0 indicates N deficiency.

Plant available water and soil moisture deficit were estimated to 900 mm soil depth for Bull Park and to 600 mm at Road Field and College Field. Plant available water (mm) was calculated using values of available water at field capacity and the depth of sparse and intensive rooting according to Bailey (1990). Estimated values of volumetric soil moisture content at field capacity for the soil textures present at the current sites were taken from Rowell (1994). The soil moisture deficit (SMD) at ear emergence was calculated as the soil moisture content (mm) at field capacity minus measured values at ear emergence. The values at ear emergence were determined from gravimetric soil moisture contents and the soil bulk densities measured at 0-300, 300-600 and 600-900 mm soil depths (Rowell 1994). Onset of water stress was assumed to occur if the SMD exceeded 50% depletion of the plant available water (Bailey 1990).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and regression analysis using SAS (18^{th} Edition). Data were checked to determine whether they conformed to the assumptions of parametric testing such as homogeneity of variance and normal distribution of residuals.

Although the literature favours linear plus exponential curves as explanatories of grain yield, there is insufficient information in the data for Road Field and College Field to allow identification of all the parameters in the model, therefore, a quadratic model was used. To compare the curves of all three sites best linear unbiased predictions (BLUPS) were used as different N levels were used in 2013 and 2014. NNI and canopy N concentration were assessed separately for site, N rate and site x N rate effects using general ANOVA treatment structure with SAS (18th Edition). Site and replication were included as random factors as treatments were unbalanced between seasons. Grain yield, grain N concentration, aboveground biomass, GAI and soil mineral N content were analysed by means of curve fitting.

Simple linear regression and split-line regression were used to investigate relationships between the individual crop characteristics outlined in Figure 1. With simple linear regression, it is assumed that errors are only in the dependent variable (Y) and the independent variable (X) is measured without error. When identifying and evaluating the underlying relationships, the possibility of error in both variables was acknowledged; although such issues are generally of more concern in food and health science research, where results may be distorted though biased answers from individuals. A number of errors in variables regression and simple linear regressions were produced and compared. As it was deemed that there was no significant difference, simple linear regressions were used for data analysis.

Stepwise multiple regression was utilised in the production of the prediction models for grain N concentration. Based on results from the analysis of relationships between individual crop characteristics at ear emergence and grain N content and yield at harvest, variables selected for inclusion in the multiple regression models were crop N content (because of its strong relationship with grain N content) and either crop biomass at ear emergence or the number of potential grains sites (because of their relationship with grain yield) and soil mineral N reserves (as a possible estimate of PANU). The stepwise procedure started with crop N content as the sole explanatory variable for grain N concentration and then introduced a variable to account for grain yield followed by soil N reserves to account for PANU. A variable was retained in the model if it gave a significant improvement ($P<0.05$) in the amount of variation accounted for. Two sets of statistical models were developed; the first using data combined from three sites over two years (Road Field 2013 and College Field and Bull Park 2014), the second using data from the two sites (College Field and Bull Park) in 2014.

3.3 **Results**

3.3.1 **Climatic Conditions**

Monthly mean and maximum temperature and rainfall data for both the 2013 and 2014 experimental growing seasons are given in Figure 3.2. The 30 year long term average (LTA) was calculated from 1981-2010.Weather data presented were obtained from the Irish National Meteorological Service, Met Éireann, which was collected from the Oak Park weather station. The 2013 season was cooler than average at the start of the year and warmer than average towards the end, with a maximum temperature of 29.5°C recorded in July. Rainfall for 2013 was well below average. The 2014 growing season began with rainfall in March and April close to the long term average. May was wetter than average and July much drier. August rainfall was almost twice the long term average. Temperature for 2014 was comparable to or just slightly higher than average.

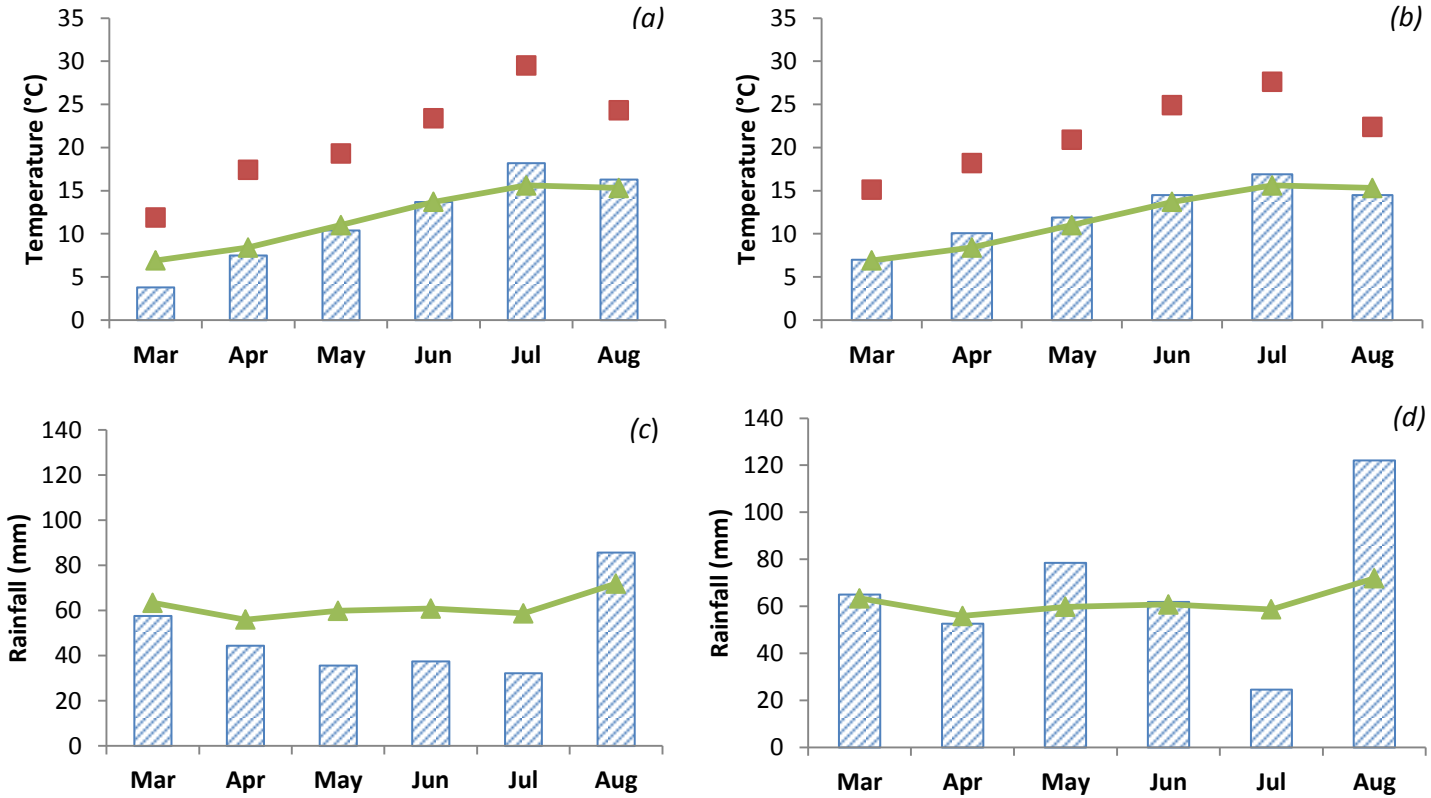

Figure 3.2. Oak Park weather data for 2013 and 2014 experimental growing seasons. Bars represent monthly mean average temperature and rainfall for 2013 (*a* and *c*) and 2014 (*b* and *d*). (■) maximum monthly temperature; (▲) 30 year (1981-2010) long term average temperature and rainfall. See Appendix 2 for further information.

Plant available water at field capacity and soil moisture deficit at ear emergence were estimated to 900 mm soil depth for Bull Park and to 600 mm at Road Field and College Field. In 2013 63% of the plant available water (PAW) in the soil profile had been depleted by anthesis (SMD was 63% of PAW) suggesting that the crop was water stressed. This is in contrast to the 2014 experimental sites which had lower levels of depleted PAW of 20% and 14% of field capacity respectively for both College Field and Bull Park. This would indicate that there were no water stress conditions in 2014 for either site.

Table 3.3. Soil moisture deficit at ear emergence in 2013 and 2014

	Road Field	**Bull Park**	**College Field**
	2013	**2014**	**2014**
Rooting depth, mm	600	>900	600
Top soil texture	Sandy loam	Sandy clay loam	Sandy loam
Sub soil texture	Sandy loam	Sandy clay loam	Sandy loam
Plant available water, mm	92	113	92
Soil moisture deficit, mm	58	16	20
% PAW depleted	63	14	21

3.3.2 Effects of N Supply on Crop Growth, Grain Yield and Grain N Concentration

As the range of N fertiliser applied in 2014 was modified from that in 2013, the experimental design across years is unbalanced. A curve fitting approach has, therefore, been taken to compare crop growth, grain yield and quality between years and sites.

3.3.2.1 Grain Yield

There was an overall effect of N rate on grain yield at each of the three experimental sites ($P<0.001$). Without fertiliser, grain yields at each of the sites were similar (around 3 t ha^{-1} at 100% DM) ($P=0.08$ for comparison of the y intercept of the three sites). However, there was a significant difference in trend ($P<0.001$) and curvature ($P<0.001$) of the yield response to N fertiliser between the three experiments.

In the 2013 Road Field experiment, application of 90 kg N ha^{-1} N increased yield from 3 t ha^{-1} (100% DM) to 4.3 t ha^{-1} but there was no further increase at any N application rates greater than that. Therefore 90 kg N ha^{-1} was estimated as the Nmax rate for grain yield at this site (N rate which at which the maximum yield was achieved), as no asymptote was achieved. By contrast optimum fertiliser N rate at Bull Park (2014) was calculated from the peak of fitted curves to be 182kg N ha^{-1} with a yield of 7.63 t ha^{-1} (100% DM). At College Field (2014) the Nmax was 170 kg N ha^{-1} producing 6.32 t ha^{-1} (100% DM).

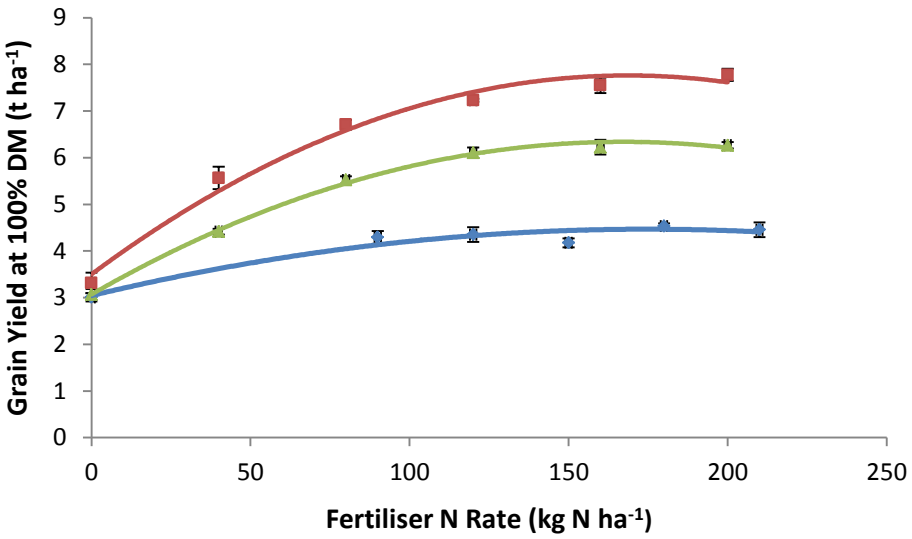

Figure 3.3. Effect of fertiliser N rate (kg N ha^{-1}) on grain yield (t ha^{-1}) in 2013 (Road Field) and 2014 (Bull Park and College Field). Values adjusted to 100% dry matter (DM). Lines show curves fitted by 2nd order polynomials to mean values for each N level; symbols represent treatment means ± SEM of 4 replicates. (■) Bull Park ($P<0.001$; $R^2 = 0.94$), (▲) College Field ($P<0.001$; $R^2 = 0.96$) (◆) Road Field ($P<0.001$; $R^2 = 0.76$).

3.3.2.2 Grain N Concentration

There was a significant ($P<0.001$) overall effect of N fertiliser rate on grain N concentration at all three sites. The response was broadly comparable at each site, with grain N concentration changing relatively little over the low range of fertiliser N applications (0 to 80 - 90 kg ha^{-1}), but increasing more rapidly in the mid-range. At Bull Park and Road Field the response tended towards an asymptote at high rates of fertiliser, but at College Field grain N concentration continued to rise (Figure 3.4). At Bull Park and College Field grain N concentration was 1.16 and 1.05 % respectively at 0 kg N ha^{-1} to a maximum of 1.74 and 1.68 % at the highest fertiliser rate of 200 kg N ha^{-1}. By contrast, grain N concentrations at Road Field were greater across all fertiliser N rates ranging from 1.34% without fertiliser to 2.65% at the highest supply (210 kg N ha^{-1}).

The y axis intercept of all three sites was significantly different indicating differences between sites in grain N% in the absence of fertiliser. Bull Park was significantly different to Road Field (P=0.035) and College Field (P=0.008), while there was also a significant difference (P<0.001) between College Field and Road Field. A two way analysis of variance for Bull Park and College Field (whose N application rates were the same) revealed overall differences between sites (P<0.01) and N rates (P<0.001), but no significant site x N rate interaction. This supports the conclusion that the response of grain N concentration to increasing fertiliser N supply was comparable at these two sites.

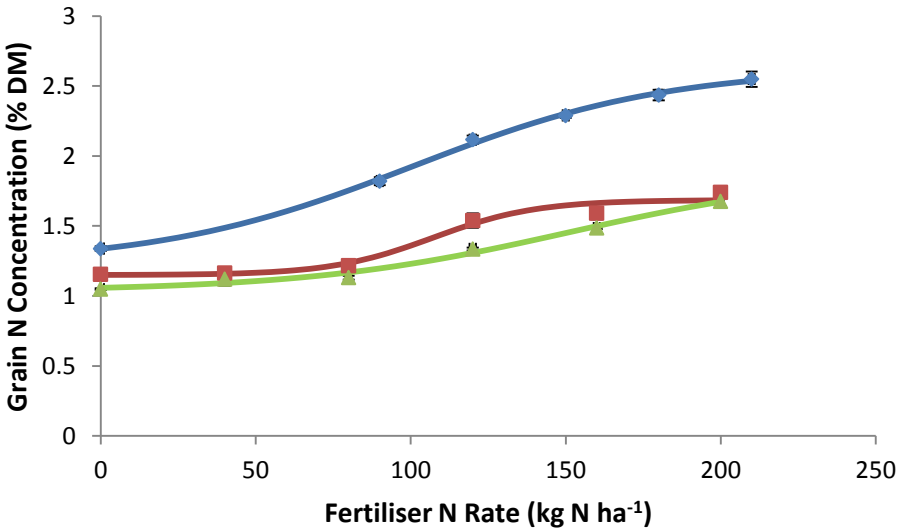

Figure 3.4. Effect of fertiliser N rate (kg N ha^{-1}) on grain N concentration (% DM) in 2013 (Road Field) and 2014 (Bull Park and College Field). Lines show curves fitted by sigmoidal to individual plot values; symbols represent treatment means ± SEM of 4 replicates. (■) Bull Park (P<0.001; R^2 = 0.87), (▲) College Field (P<0.001; R^2 = 0.93) (◆) Road Field (P<0.001; R^2 =0.78).

3.3.2.3 Aboveground Biomass

There was a significant increase in aboveground biomass in response to N fertiliser application at each growth stage and at each site ($P<0.001$; Figure 3.5). A linear plus exponential model using fertiliser N application rate as the explanatory variable accounted for more than 61 % of the variation in biomass in all cases and more than 90% in 7 out of the 9 site-crop growth stage combinations.

The greatest response occurred at Bull Park and College Field in 2014 and the least at Road Field 2013. In the absence of N fertiliser aboveground biomass at ear emergence (GS 59) was comparable between sites (4.6-4.7 t ha^{-1}). There was a 119% increase in biomass as fertiliser N application was increased from zero N to the optimum N rate at Bull Park, a 109% increase at College Field, but only a 43% increase at Road Field. The Nmax were 169, 147 and 90 kg N ha^{-1} for Bull Park, College Field and Road Field respectively giving a biomass at GS 59 of 10.3, 9.6 and 6.3 t ha^{-1}. Road Field biomass Nmax was estimated at 90 kg N ha^{-1} as higher levels of fertiliser N gave no significant increase in biomass.

The differences in crop biomass between sites at GS59 were largely the result of differences in growth after flag leaf emergence. For example, at both GS31 and GS39, aboveground biomass for Bull Park and Road Field at an N rate of 170 kg N ha^{-1} (the optimum at GS59) differed by only 0.5 t ha^{-1}, whereas at GS59 the difference had increased to 3.6 t ha^{-1}.

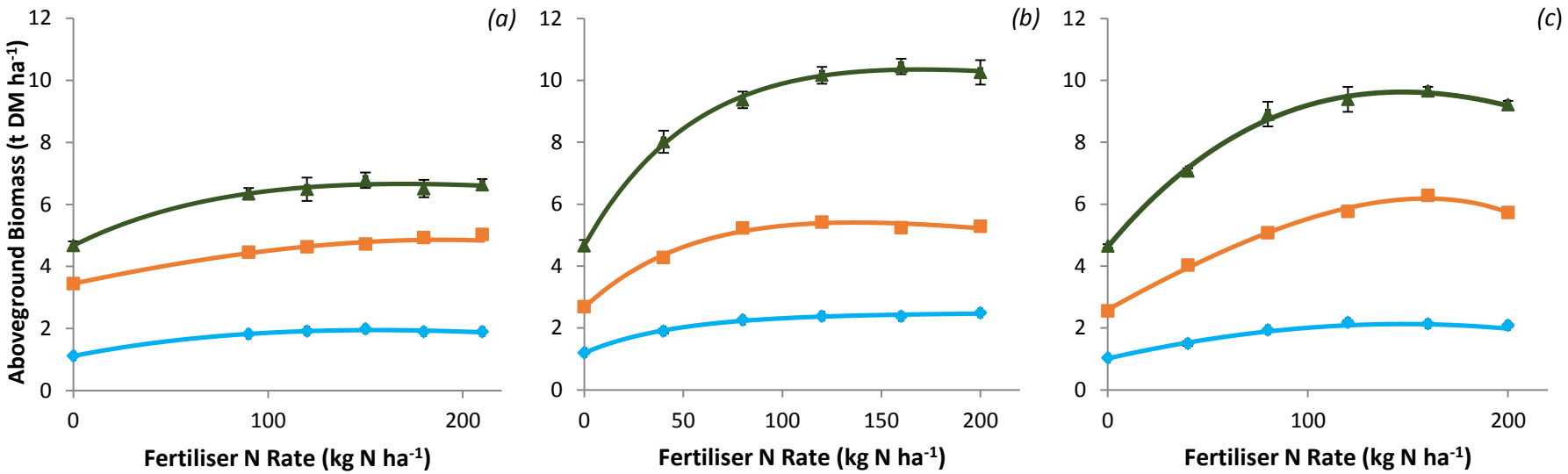

Figure 3.5. Effect of fertiliser N rate (kg N ha^{-1}) on aboveground biomass (t DM ha^{-1}) accumulation in (*a*) Road Field 2013, (*b*) Bull Park 2014 and (*c*) College Field 2014 at GS 31 (◆), GS 39 (■) and GS 59 (▲). Lines show curves fitted to individual plot values using a linear plus exponential model; symbols represent treatment means ± SEM of 4 replicate plots. Road Field: *(a)* (GS31- $P<0.001$; $R^2 = 0.92$) (GS 39 - $P<0.001$; $R^2 = 0.88$) (GS 59 - $P<0.001$; $R^2 = 0.61$). Bull Park: *(b)* (GS31- $P<0.001$; $R^2 = 0.95$) (GS 39 - $P<0.001$; $R^2 = 0.95$) (GS 59 - $P<0.001$; $R^2 = 0.91$). College Field: *(c)* (GS31- $P<0.001$; $R^2 = 0.92$) (GS 39 - $P<0.001$; $R^2 = 0.97$) (GS 59 - $P<0.001$; $R^2 = 0.91$).

3.3.2.4 Green Area Index

The application of fertiliser N increased ($P<0.001$) the green area index (GAI) at each crop growth stage and at all three experimental sites across both years of the study (Figure 3.6). The greatest response occurred at Bull Park and College Field in 2014 and the least at Road Field 2013. In the absence of N fertiliser GAI at ear emergence (GS59) was 1.2, 1.6 and 2.4 for Bull Park, College Field and Road Field respectively. This was increased to a maximum of 6.3 at 170 kg N ha^{-1} at Bull Park and 7.1 at 200 N ha^{-1} at College Field, but only 4.9 at 150 N ha^{-1} at Road Field. The dynamics of canopy growth also appeared to differ between sites. At GS31 and GS39 GAI was broadly comparable across the N fertiliser treatments at each site, but between GS39 and 59 the increase in GAI at Bull Park and College Field was greater than that at Road Field. Thus, with fertiliser rates close to those giving the maximum GAI for a given growth stage GAI increased by 2.3 between GS39 and 59 at Bull Park, 2.5 at College Field and only 0.5 at Road Field.

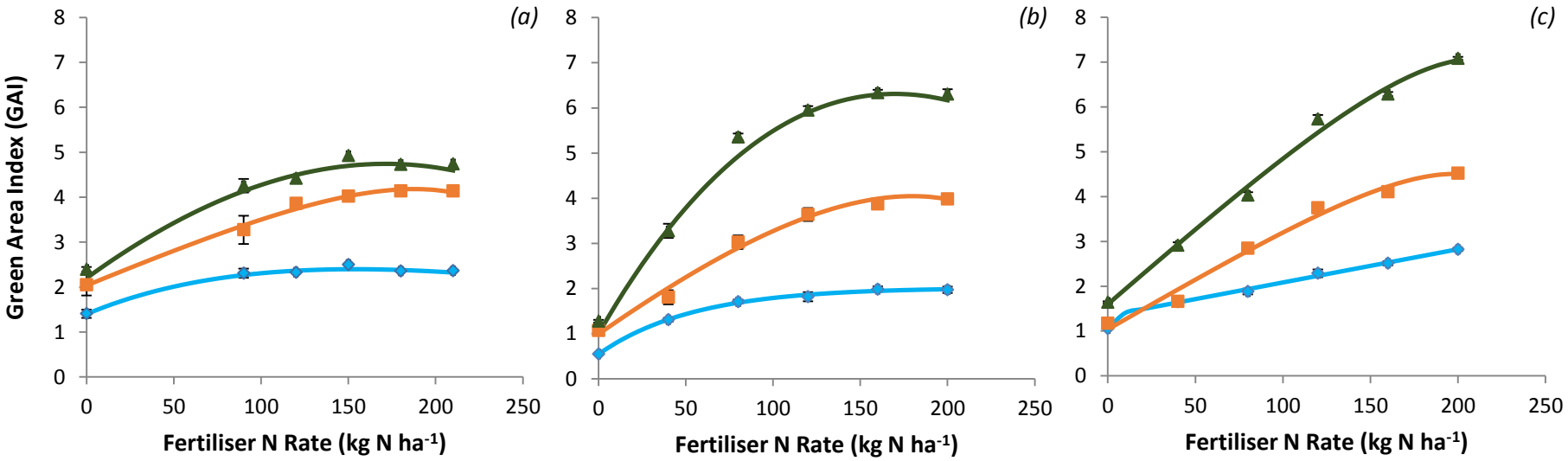

Figure 3.6. Effect of fertiliser N rate (kg N ha^{-1}) on green area index (GAI) in (a) Road Field 2013, (b) Bull Park 2014 and (c) College Field 2014 at GS 31 (◆), GS 39 (■) and GS 59 (▲). Lines show curves fitted to individual plot values using a linear plus exponential model; symbols represent treatment means ± SEM of 4 replicate plots Road Field: *(a)* (GS31- P<0.001; R^2 = 0. 63) (GS 39 - P<0.001; R^2 =0.60) (GS 59 - P<0.001; R^2 = 0. 92). Bull Park: *(b)* (GS31- P<0.001; R^2 = 0.93) (GS 39 - P<0.001; R^2 = 0.94) (GS 59 - P<0.001; R^2 = 0.98). College Field: *(c)* (GS31- P<0.001; R^2 = 0.95) (GS 39 - P<0.001; R^2 = 0.98) (GS 59 - P<0.001; R^2 = 0.97).

3.3.2.5 Canopy N Concentration and NNI

There was a significant increase in canopy N concentration (N % aboveground DM) and NNI in response to N fertiliser application at GS 59 at all three sites ($P<0.001$; Table 3.4). A linear model using fertiliser N application rate as the explanatory variable accounted for more than 90% in of variation in N concentration and NNI. The highest N concentration (2.14 % DM) and NNI (1.56) was achieved at the Road Field site in 2013 at 210 kg N ha^{-1}. At the Road Field site (2013), all N fertiliser applications from 120 kg N ha^{-1} and above resulted in crops with luxury N uptake as illustrated by NNI values presented in Table 3.4. Optimum crop N status was reached at 160 and 180 kg N ha^{-1} at the College Field and Bull Park sites in 2014 respectively.

Table 3.4. The effect of fertiliser N rate (kg N ha^{-1}) on the mean canopy N concentration (N % DM) and nitrogen nutrition index (NNI) of the three experimental sites at GS 59 for Road Field (2013) and Bull Park and College Field (2014).

Site	N Rate		Canopy N (% DM)		NNI	
Road	**0**		0.87		0.68	
	90		1.38		0.84	
	120		1.62		1.02	
	150		1.76		1.22	
	180		2.02		1.41	
	210		2.14		1.56	
		df	**P**	**LSD**	**P**	**LSD**
N Rate		5	<.001	0.188	<.001	0.098
Residual df		18				
Bull	**0**		0.79		0.51	
	40		0.86		0.55	
	80		1.03		0.66	
	120		1.35		0.87	
	160		1.48		0.95	
	200		1.65		1.06	
College	**0**		0.73		0.52	
	40		0.80		0.57	
	80		0.94		0.67	
	120		1.27		0.91	
	160		1.45		1.03	
	200		1.73		1.23	
		df	**P**	**LSD**	**P**	**LSD**
N Rate (N)		5	<.001	0.063	<.001	0.042
Site (S)		1	0.039	0.036	<.001	0.024
S * N		5	0.111	ns	0.003	0.059
Residual df		36				

3.3.2.6 Soil Mineral N Content

There was an overall effect of fertiliser N rate on soil mineral N content at GS59 (measured to 60 cm soil depth) at each of the three experimental sites (P<0.001) (Figure 3.7). When no fertiliser N was applied, soil mineral N contents for the three sites were approximately 20-30 kg N ha^{-1}. Despite the small range at 0 kg N ha^{-1}, there was a significant difference between all three sites (intercept P<0.001). There was also a significant difference in both trend (P<0.001) and curvature (P<0.001) of the soil N contents in response to N fertiliser at all three sites. In 2013, Road Field soil N reserves increased steeply with the application of fertiliser N from 20 kg N ha^{-1} to just over 100 kg N ha^{-1} at the maximum fertiliser N application of 210 kg N ha^{-1}. Both of the 2014 sites performed in a comparable manner with each other, but differed from Road Field in 2013. At both Bull Park and College Field there was relatively little change in soil N content with any fertiliser application up to 120 kg fertiliser N ha^{-1}. The application of 200 kg fertiliser N ha^{-1} increased soil N content to around 48 N ha^{-1} at Bull Park and 33 kg N ha^{-1} at College Field representing an increase of 15 kg and 7 kg N ha^{-1} respectively over that with no N fertiliser application.

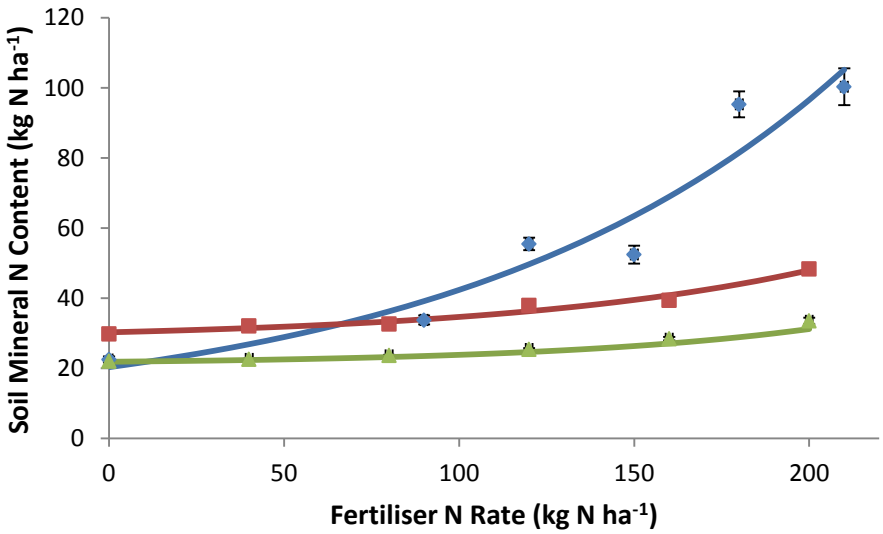

Figure 3.7. Effect of fertiliser N rate (kg N ha^{-1}) on soil mineral N content (kg N ha^{-1} 0-60 cm soil depth) at GS59. Lines show curves fitted to individual plot values using a 2nd order polynomial model; symbols represent treatment means ± SEM of 4 replicate plots (◆) Road Field 2013 (P<0.001; R^2 = 0.83). (■) Bull Park 2014 (P<0.001; R^2 = 0.85), (▲) College Field 2014 (P<0.001; R^2 = 0.69).

3.3.3 **Variation in Grain N Concentration and its Components**

3.3.3.1 Determination of Grain N Concentration

The relationship between grain N concentration and grain N content (kg N ha^{-1}) is shown in Figure 3.8. Linear regression analysis of data pooled from the three site-seasons indicated that grain N content explained only a relatively small amount of the variation in grain N% ($P<0.001$; $R^2 = 0.44$). Interestingly, as evident from the graph, there are two prominent individual relationships present. Thus two separate models were fitted to the data sets; (*a)* represents the Road Field experimental site with a significant linear relationship ($P<0.001$; $R^2 = 0.93$) and *(b)* represents the combined data from Bull Park and College Field analysed using split-line regression ($R^2 = 0.89$). The split-line regression accounted for a slightly greater amount of the variation than a simple linear regression ($R^2 = 0.86$). The breakpoint was at a grain N content of 53.7 kg N ha^{-1} (95% confidence interval 41.2 to 64.4 kg N ha^{-1}). At N contents below the breakpoint, grain N concentration changed little with increasing N content, but above it, the N concentration increased.

Figure 3.9 illustrates the relationship between grain N concentration and grain yield (t ha^{-1} at 100% DM). When data from the three site-seasons were combined no significant ($P=0.71$; $R^2 = 0.002$) overall relationship was found between grain yield and grain N concentration. As for Figure 3.9, the data for different site-seasons divided into two distinct groups. There was a significant linear relationship ($P<0.001$) between grain yield and grain N concentration for the Road Field site that accounted for 60% of variation in grain N concentration. When data were combined from Bull Park and College Field, grain yield accounted for 63% of the variation in grain N concentration using split-line regression analysis. There appeared to be little change in grain N concentration as yield increased up to the breakpoint of 4.83 t ha^{-1} (95% confidence interval; 3.53 to 5.45 t ha^{-1}). Thereafter, N concentration increased with yield.

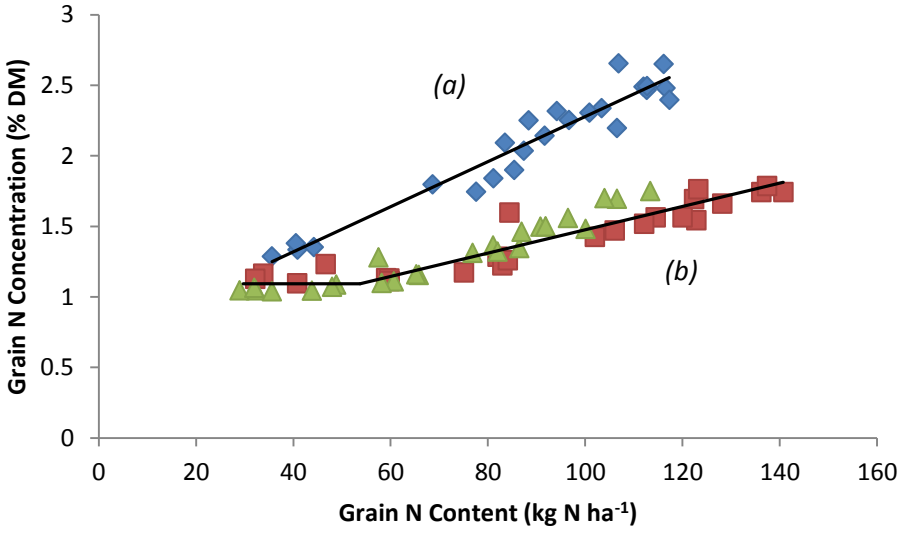

Figure 3.8. Relationship between grain N content (kg N ha^{-1}) and grain N concentration (N %) where *(a)* represents Road Field data 2013 (◆), ($P<0.001$; $R^2 = 0.93$), *(b)* represents combined 2014 data from Bull Park (■), and College Field (▲) ($P<0.001$; $R^2 = 0.89$). Data points represent individual plot values.

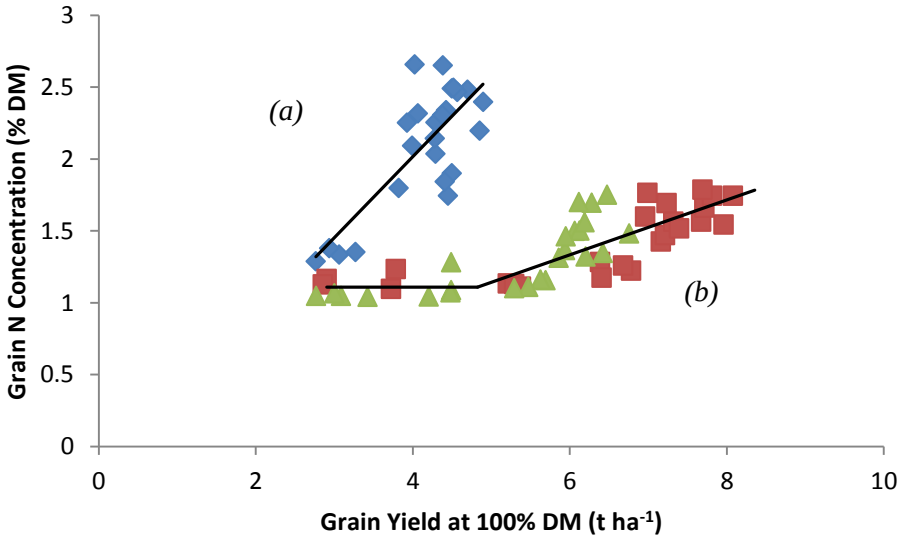

Figure 3.9. Relationship between grain yield (t ha^{-1} at 100% DM) and grain N concentration where *(a)* represents Road Field data (◆), ($P<0.001$; $R^2 = 0.60$), *(b)* represents combined Bull Park (■) and College Field (▲) data ($P<0.001$; $R^2 = 0.63$). Data points represent individual plot values.

3.3.3.2 Determination of Grain N Content

Grain N content is the product of two sub-components, the total crop N content and the N harvest index (NHI). A very strong, relationship ($P<0.001$) was found between grain N content (kg N ha^{-1}) and crop N content (kg N ha^{-1}) which accounted for a high level of variation in grain N content ($R^2 = 0.96$) (Figure 3.10). The relationship was observed at all three site-seasons when all data were combined. It also spanned a wide range of crop N contents from 40 kg N ha^{-1} to 200 kg N ha^{-1}. By contrast, there was no significant relationship between grain N content (kg N ha^{-1}) and NHI ($P=0.79$; $R^2 = 0.001$) (Figure 3.11). Despite the large range of grain N contents (20 - 140 kg N ha^{-1}) across treatments and sites, there was little difference in NHI with most values being within the range 60-80%. Thus, across the sites and N fertiliser N treatments the majority of the variation observed in grain N content was associated with variation in total N content of the crop and not NHI.

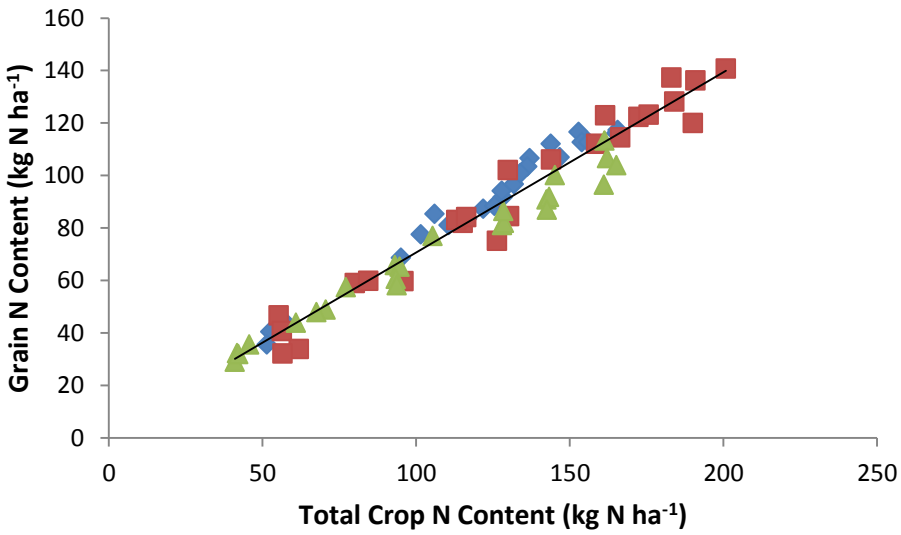

Figure 3.10. Relationship between total crop N content (kg N ha^{-1}) and grain N content (kg N ha^{-1}) ($P<0.001$; $R^2 = 0.96$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

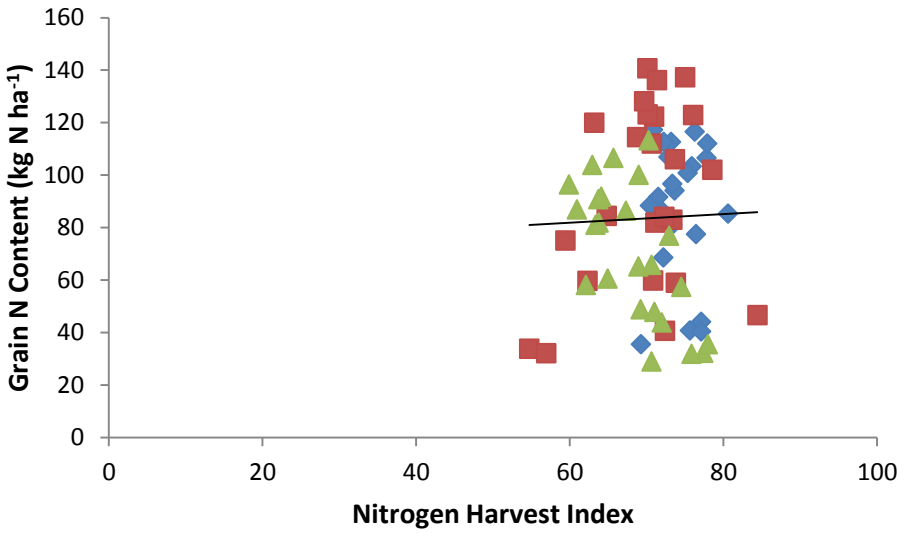

Figure 3.11. Relationship between nitrogen harvest index (NHI) and grain N content (kg N ha^{-1}) (P=0.79; R^2 = 0.001). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

3.3.3.3 Determination of harvest crop N content

A highly significant relationship (P<0.001, R^2 = 0.94) was found between total aboveground N content at harvest and canopy N content at GS 59 (Figure 3.12). This relationship was observed for GS 59 canopy N contents ranging from 30 kg N ha^{-1} to 175 kg N ha^{-1} and a single relationship explained the variation in above-ground N content in data pooled from the three site-seasons. Conversely, there was no relationship (P=0.35) evident between total crop N content at harvest and post-anthesis N uptake (PANU) (Figure 3.13). Thus PANU made relatively little contribution to the variation in above-ground N content at harvest across the sites and N fertiliser treatments in this study.

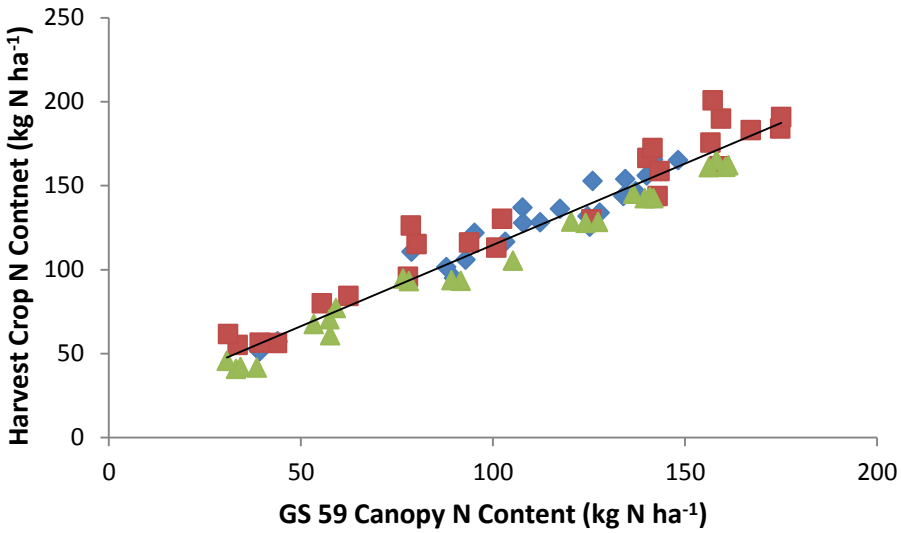

Figure 3.12. Relationship between GS 59 canopy N content (kg N ha^{-1}) and above-ground N content at harvest (kg N ha^{-1}) ($P<0.001$; $R^2 = 0.94$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

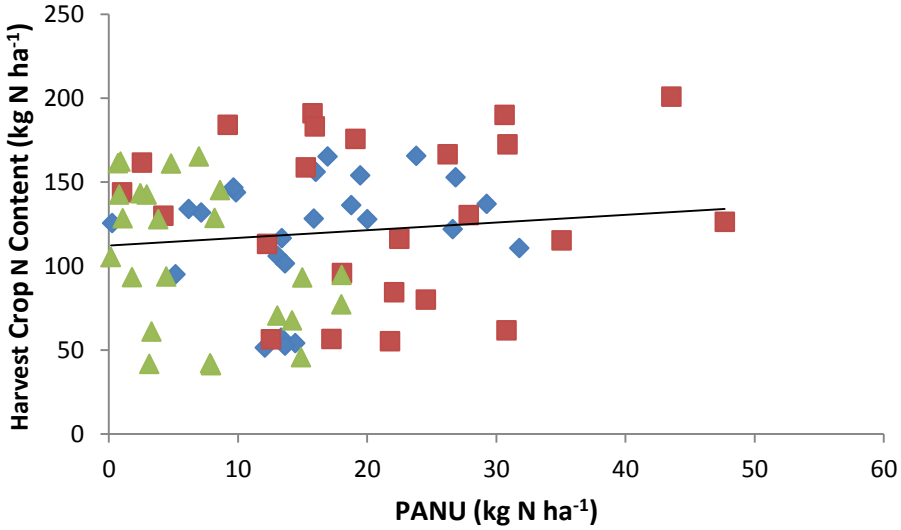

Figure 3.13. Relationship between PANU (kg N ha^{-1}) and harvest crop N content (kg N ha^{-1}) ($P=0.35$; $R^2 = 0.013$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

3.3.3.4 Determination of Grain Yield

Grain yield is the product of the number of grains per unit area and the mean grain weight. Grain yield (t DM ha^{-1}) was positively associated with harvest grain number (m^{-2}) displaying a highly significant ($P<0.001$) relationship that explained 94% of the variation in yield when the combination of sites and years were included (Figure 3.14). This relationship occurred over a range of grain numbers from 6,600 to 18,600 grains m^{-2}. Grain yield (t DM ha^{-1}) also illustrated a significant relationship ($P<0.001$) with grain weight (mg) although mean grain weight accounted for a smaller proportion of the variation in yield ($R^2 = 0.41$) than grain number (Figure 3.15).

A relatively modest relationship ($P<0.001$, $R^2 = 0.45$) was found between grain number (m^{-2}) and the number of potential grain sites at GS 59 (ear fully emerged) when data from individual sites were pooled (Figure 3.16).

Yield can also be described quantitatively as the product of aboveground biomass at harvest and the harvest index (HI). Grain yield (t DM ha^{-1}) displayed a strong positive association with harvest biomass (kg DM ha^{-1}) ($P<0.001$, $R^2 = 0.83$) (Figure 3.17) when data from the three site-seasons were pooled. The relationship was observed over a wide range of biomass values from 5000 kg DM ha^{-1} to 18000 kg DM ha^{-1}. Fitting separate lines with a common slope, but allowing intercepts to vary, to data from individual sites significantly ($P<0.001$) increased the amount of variation in yield accounted for to 93%. Grain yield (t DM ha^{-1}) was not related to harvest index (HI) when data from each of the sites was pooled ($P=0.16$, $R^2 = 0.028$), but weak negative relationships were found when separate lines were fitted to data for individual sites (with common slope and different intercepts) (Figure 3.18). However, the total amount of variation accounted for increased to only 43%.

There was a strong relationship between harvest biomass (kg DM ha^{-1}) and GS 59 biomass (kg DM ha^{-1}) ($P<0.001$, $R^2 = 0.79$) when data for all sites were pooled (Figure 3.19). Separate lines with a common slope but different intercepts resulted in only a small improvement in the amount of variation accounted for (additional 5%; $P<0.001$).

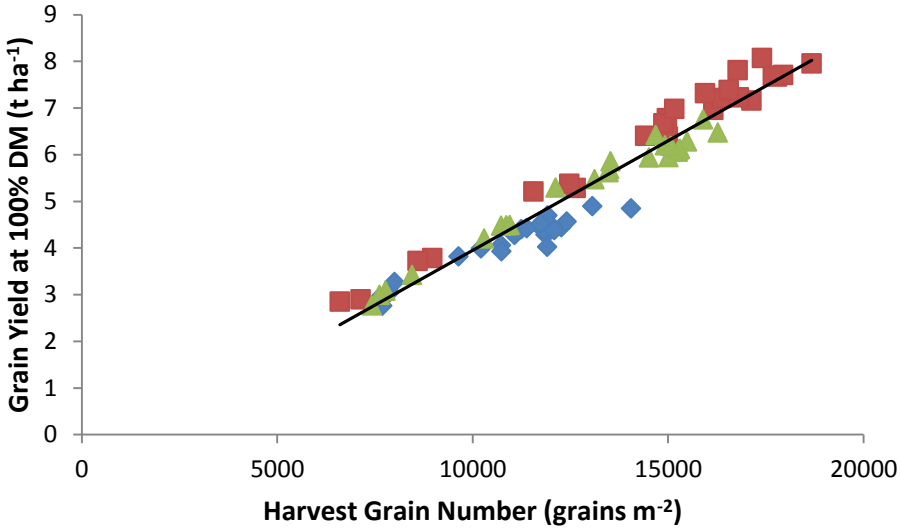

Figure 3.14. Relationship between final harvest grain number m^{-2} and final grain yield (t ha^{-1} at 100% DM) ($P<0.001$; $R^2 = 0.94$) (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

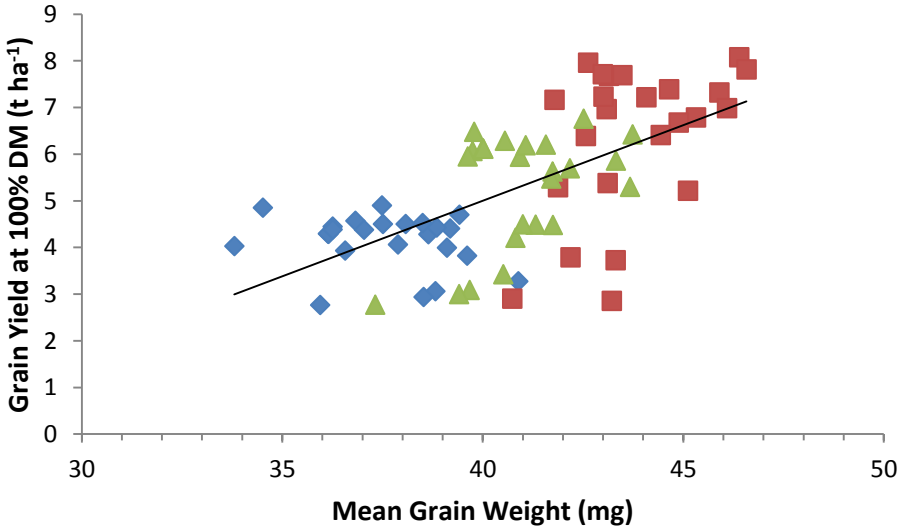

Figure 3.15. Relationship between mean grain weight and grain yield (t ha^{-1} at 100% DM) ($P<0.001$; $R^2 = 0.41$) (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

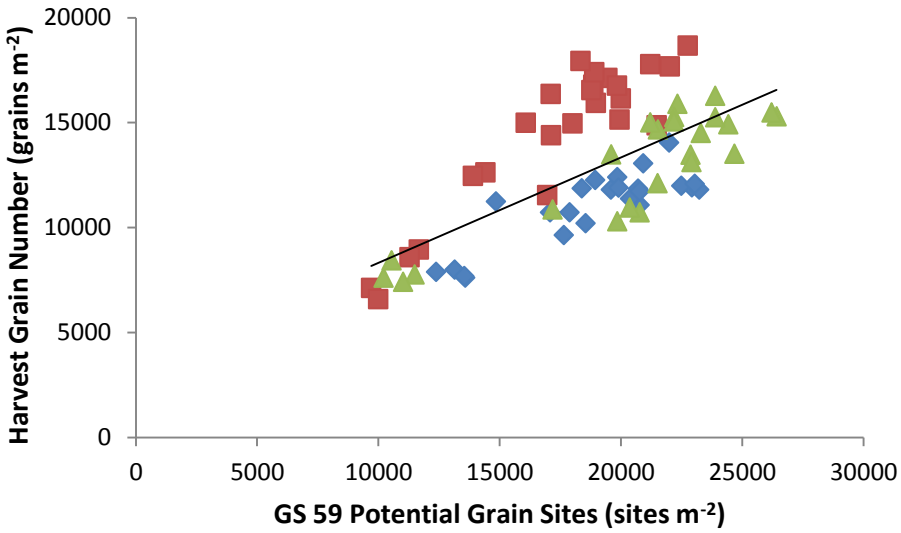

Figure 3.16. Relationship between potential grain site numbers (m^{-2}) at GS 59 and harvest grain number m^{-2}) ($P<0.001$; $R^2 = 0.45$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

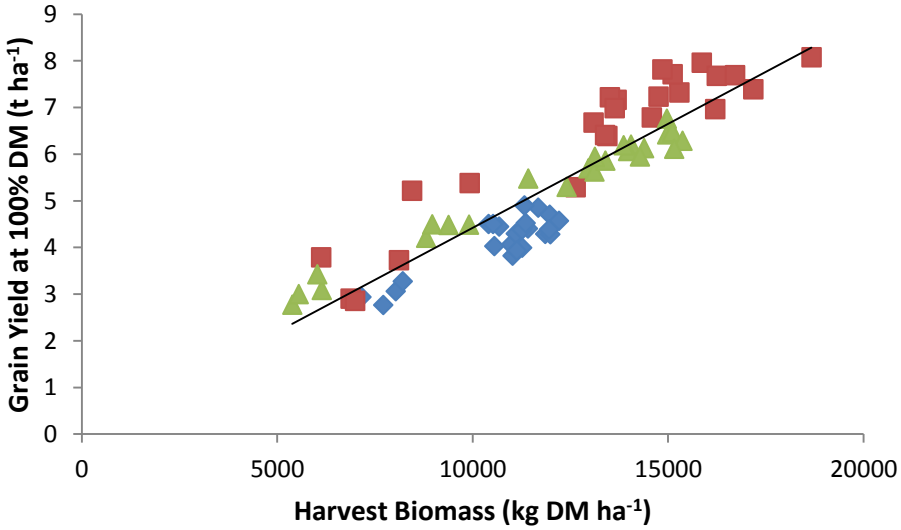

Figure 3.17. Relationship between harvest biomass (kg DM ha^{-1}) and grain yield (t ha^{-1} at 100% DM) ($P<0.001$; $R^2 = 0.83$) (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

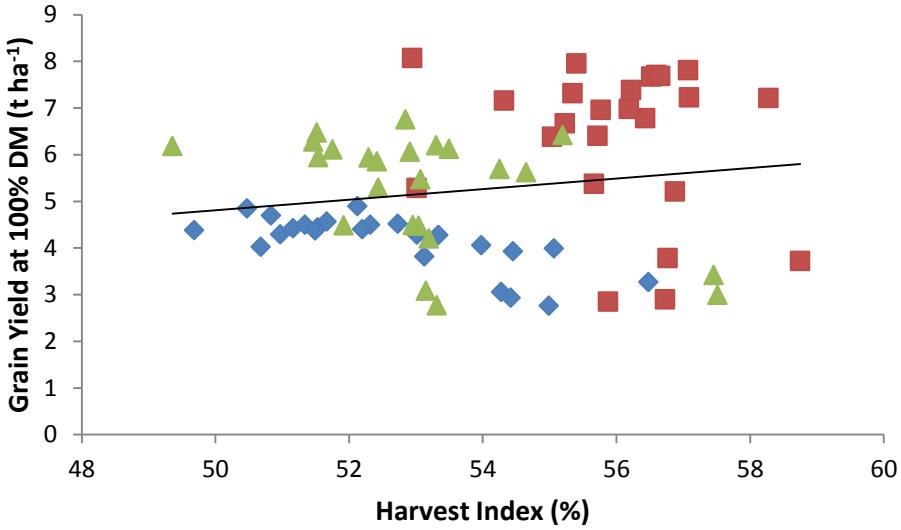

Figure 3.18. Relationship between harvest index and grain yield (t ha^{-1} at 100% DM) (P=0.16; R^2 = 0.028) (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

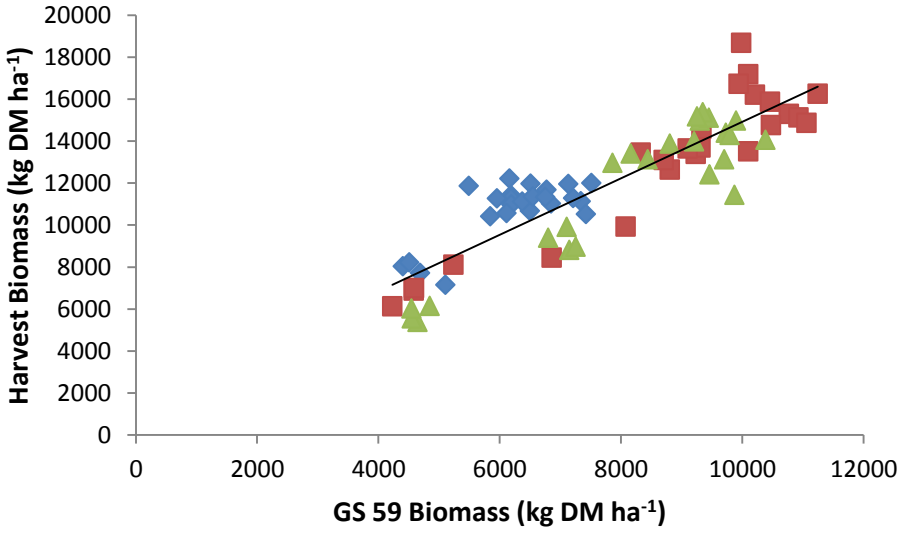

Figure 3.19. Relationship between GS 59 biomass (kg DM ha^{-1}) and harvest biomass (kg DM ha^{-1}) (P<0.001; R^2 = 0.79). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

3.3.3.5 Estimating Grain N Content, Yield and PANU from GS 59 Measurements

To determine whether GS 59 measurements could be used to estimate the grain N content and grain yield (the two main components of grain N concentration), regression analysis was used to examine the relationships between grain N content and canopy N content at GS 59, grain yield and GS 59 measurements of above ground biomass and potential grain number. There was a significant relationship ($P<0.001$) evident between grain N content (kg N ha^{-1}) and GS 59 canopy N content (kg N ha^{-1}) which accounted for 90% of the variation in grain N (Figure 3.20). Thus a single relationship provided a good description of the data from across three site-season experiments. Although grain yield (t ha^{-1} at 100% DM) was significantly related ($P<0.001$) to the number of potential grain sites (m^{-2}) the relationship was weak and accounted for only a small proportion of the variation ($R^2 = 0.31$) when data from the three sites were pooled (Figure 3.21). It is clear that the relationship between grain yield and potential grain number differed between sites with yield increasing to a greater extent with a given increase in potential grain number at Bull Park compared to Road Field and College Field. By contrast, a single relationship between grain yield (t ha^{-1} at 100% DM) and GS 59 biomass (kg DM ha^{-1}) explained 88% of variation in yield across sites and years (Figure 3.22). The correlation was observed from 4,200 kg DM ha^{-1} up to the maximum biomass level of 11,200 kg DM ha^{-1}. There was no significant relationship between PANU (kg N ha^{-1}) and soil mineral N content at GS59 (kg N ha^{-1}) for any of the sites individually or when data were combined (Figure 3.23).

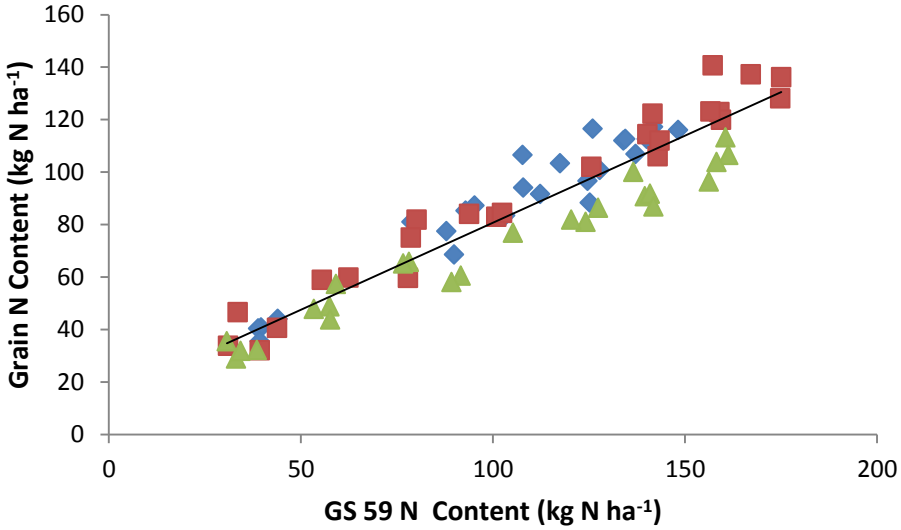

Figure 3.20. Relationship between GS 59 canopy N content (kg N ha^{-1}) and grain N content (kg N ha^{-1}) ($P<0.001$; $R^2 = 0.90$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

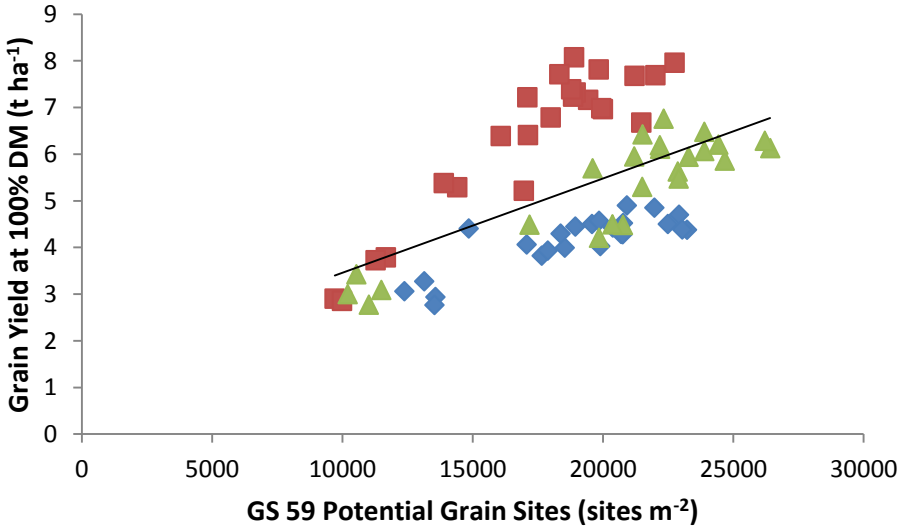

Figure 3.21. Relationship between GS 59 potential grain site numbers (m^{-2}) and grain yield (t ha^{-1} at 100% DM) ($P<0.001$; $R^2 = 0.31$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

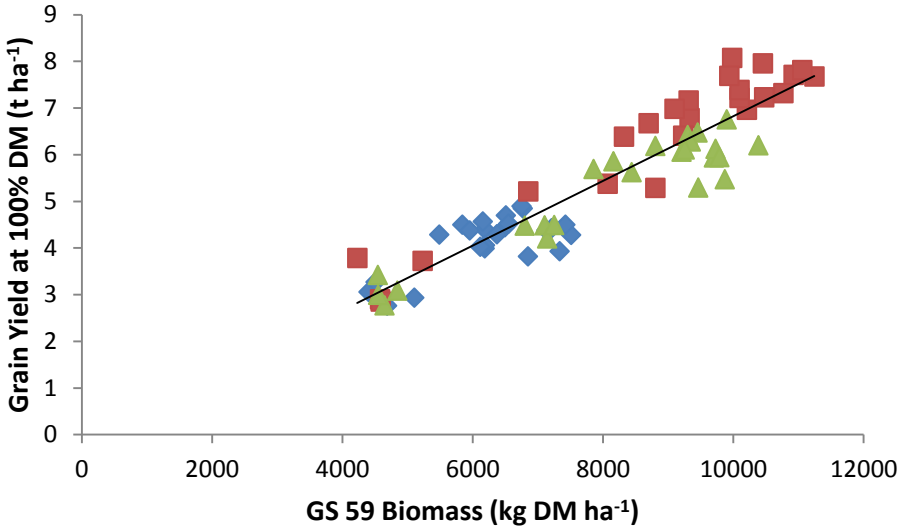

Figure 3.22. Relationship between GS 59 biomass (kg DM ha^{-1}) and grain yield (t ha^{-1} at 100% DM) ($P<0.001$; $R^2 = 0.88$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

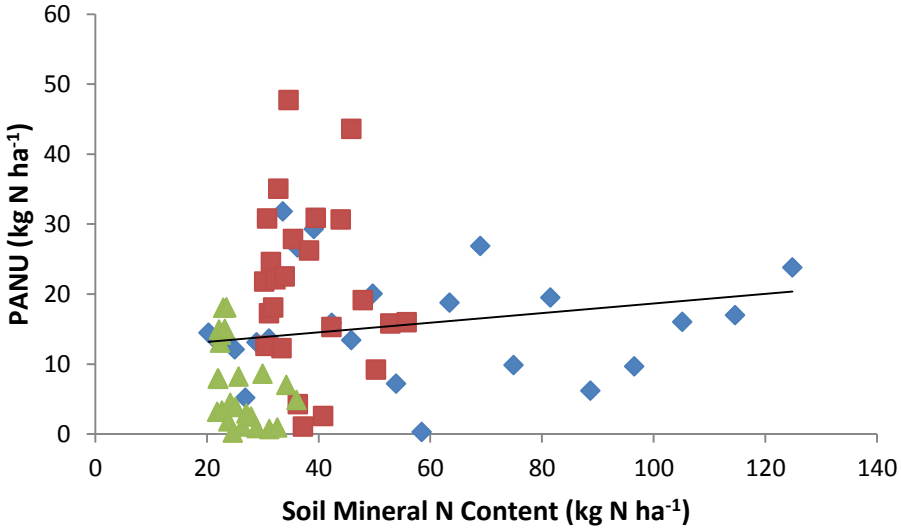

Figure 3.23. Relationship between soil mineral N content (kg N ha^{-1}) at GS 59 measured to 60 cm soil depth and PANU (kg N ha^{-1}) ($P=0.377$; $R^2 = 0.106$). (◆) Road Field, (■) Bull Park, (▲) College Field. Each point represents an individual plot value.

3.3.4 Multiple Regression Models for Estimating Grain N Concentration

Using those GS 59 measurements that accounted for the largest proportion of the variation in grain N content and yield (above) as explanatory variables, several multiple regression models were constructed in a stepwise approach to explain the variation observed in grain N concentration across sites and N fertiliser treatments. The first series of models used data for all three sites. In the second series data from Road Field 2013 were excluded on the grounds that likely soil water stress substantially altered the relationships between grain N concentration and both yield and grain N content (Figures 3.8 and 3.9).

Table 3.5. Model 1. Stepwise multiple regression of grain N concentration against the explanatory variables N content, biomass and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from all three experimental sites across two years (2013 and 2014).

Model	Explanatory variable	F pr.	Variation (%)	Equation
1a	GS 59 N Content	<0.001	31.4	$= 0.951 + 0.00620 * (N\ Content)$
1b	GS 59 N Content + GS 59 Biomass	<0.001 <0.001	80	$= 1.9251 + 0.014177 * (N\ Content) - 0.0002335 * (Biomass)$
1c	GS 59 N Content + GS 59 Biomass + GS 59 Soil Mineral N Content	<0.001 <0.001 <0.001	87.3	$= 1.427 + 0.00920 * (N\ Content) - 0.0001461 * (Biomass) + 0.00842 * (Soil\ Mineral\ N\ Content)$

Table 3.6. Model 2. Stepwise multiple regression of grain N concentration against the explanatory variables N content, number of potential grain sites and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from all three experimental sites across two years (2013 and 2014).

Model	Explanatory variable	F pr.	Variation (%)	Equation
2a	GS 59 N Content	<0.001	31.4	$= 0.951 + 0.00620\ (N\ Content)$
2b	GS 59 N Content + Potential Grain Sites	<0.001 <0.659	30.6	$= 1.035 + 0.00672 * (N\ Content) - 0.0000073 * (Potential\ Grain\ Sites)$
2c	GS 59 N Content + Potential Grain Sites + GS 59 Soil Mineral N Content	0.021 0.713 <.001	77.1	$= 0.669 + 0.002337 * (N\ Content) - 0.00000354 * (Potential\ Grain\ Sites) + 0.01523 * (Soil\ Mineral\ N\ Content)$

Table 3.7. Model 3. Stepwise multiple regression of grain N concentration against the explanatory variables N content, biomass, number of potential grain sites and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from all three experimental sites across two years (2013 and 2014).

Model	Explanatory variable	F pr.	Variation (%)	Equation
3a	GS 59 N Content	<0.001	31.4	$= 0.951 + 0.00620\ (N\ Content)$
3b	GS 59 N Content + GS 59 Biomass	<0.001 <0.001	80	$= 1.9251 + 0.014177 * (N\ Content) - 0.0002335 * (Biomass)$
3c	GS 59 N Content + GS 59 Biomass + Potential Grain Sites	<0.001 <0.001 0.064	80.7	$= 1.760 + 0.013212.* (N\ Content) - 0.0002403 * (Biomass) - 0.00001684 * (Potential\ Grain\ Sites)$
3d	GS 59 N Content + GS 59 Biomass + Potential Grain Sites + GS 59 Soil Mineral N Content	<0.001 <0.001 0.053 <.001	87.8	$= 1.300 + 0.00849 * (N\ Content) - 0.0001534 * (Biomass) - 0.00001400 * (Potential\ Grain\ Sites) + 0.00826 *$ *(Soil Mineral N Content)*

Table 3.8. Model 4. Stepwise multiple regression of grain N concentration against the explanatory variables N content, biomass and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from Bull Park and College Field 2014.

Model	Explanatory variable	F pr.	Variation (%)	Equation
4a	GS 59 N Content	<0.001	84.5	$= 0.8550 + 0.004742\ (N\ Content)$
4b	GS 59 N Content + GS 59 Biomass	<0.001 <0.001	91	$= 1.1422 + 0.007120 * (N\ Content) - 0.0000631 * (Biomass)$
4c	GS 59 N Content + GS 59 Biomass + GS 59 Soil Mineral N Content	<0.001 <0.001 0.013	87.4	$= 1.0494 + 0.006589 * (N\ Content) - 0.0000608 * (Biomass) + 0.00415 * (Soil\ Mineral\ N\ Content)$

Table 3.9. Model 5. Stepwise multiple regression of grain N concentration against the explanatory variables N content, number of potential grain sites, and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from Bull Park and College Field in 2014.

Model	Explanatory variable	F pr.	Variation (%)	Equation
5a	GS 59 N Content	<0.001	84.5	$= 0.951 + 0.00620\ (N\ Content)$
5b	GS 59 N Content + Potential Grain Sites	<0.001 0.002	87.1	$= 1.0050 + 0.005610 * (N\ Content) - 0.00001273 * (Potential\ Grain\ Sites)$
5c	GS 59 N Content + Potential Grain Sites + GS 59 Soil Mineral N Content	<0.001 0.040 0.655	86.9	$= 0.962 + 0.005373 * (N\ Content) - 0.00001117 * (Potential\ Grain\ Sites) - 0.00123 * (Soil\ Mineral\ N\ Content)$

Table 3.10. Model 6. Step-wise multiple regression of grain N concentration against the explanatory variables N content, biomass, number of potential grain sites, and soil mineral N content measured at GS 59. The proportion of the total variation accounted for and the statistical significance of introducing each explanatory variable are shown. Based on data from Bull Park and College Field in 2014.

Model	Explanatory variable	F pr.	Variation (%)	Equation
6a	GS 59 N Content	<0.001	84.5	$= 0.8550 + 0.00620\ (N\ Content)$
6b	GS 59 N Content + GS 59 Biomass	<0.001 <0.001	91	$= 1.1422 + 0.007120 * (N\ Content) - 0.0000631 * (Biomass)$
6c	GS 59 N Content + GS 59 Biomass + Potential Grain Sites	<0.001 <0.001 0.087	91.4	$= 1.1787 + 0.007242 * (N\ Content) - 0.0000553 * (Biomass) - 0.00000613 * (Potential\ Grain\ Sites)$
6d	GS 59 N Content + GS 59 Biomass + Potential Grain Sites + GS 59 Soil Mineral N Content	<0.001 <0.001 0.924 0.078	91.8	$= 1.0553 + 0.006616 * (N\ Content) - 0.0000603 * (Biomass) - 0.00000045 * (Potential\ Grain\ Sites) + 0.00401 * (Soil\ Mineral\ N\ Content)$

Because of its strong relationship with grain N content canopy N content at GS 59 was included as the first variable in all models. Model 1, (Table 3.3), was constructed from data compiled from three experimental sites across two years of study. Canopy N content (kg N ha^{-1}) at GS 59 on its own accounted for 31.4% of the variation in grain N concentration. When GS 59 biomass (kg DM ha^{-1}) was added to the analysis as a second explanatory variable its effect was significant (P<0.001) and the level of variance accounted for was increased to 80%. With the subsequent addition of GS 59 soil mineral N content (kg N ha^{-1}) (P<0.001) there was a further increase in the amount of variation accounted for (87.3%).

Model 2 (Table 3.4) again used GS 59 N content (kg N ha^{-1}) as the variable to estimate grain N content, but the number of potential grain sites at GS59 as the

predictor of grain yield. However, potential grain sites did not have a significant effect (P=0.659) and the amount of variation in grain N concentration accounted for was only 30.6%. Finally, the inclusion of GS 59 soil mineral N content (P<0.001), increased the level of variation explained to 77.1%.

Model 3 (Table 3.5) used GS 59 canopy N content (kg N ha^{-1}) in conjunction with GS 59 aboveground biomass (kg DM ha^{-1}) and potential grain sites (m^{-2}). This model, accounted for 80.7% of grain N concentration variation. Although, the addition of potential grain sites was not significant (P=0.064), the inclusion of GS 59 soil N content (P<0.001) did increase the percentage of variation accounted for (87%).

Tables 3.6 and 3.7 show the analyses of data from two sites, Bull Park and College Field 2014, using the same explanatory variables as used in models 1 and 2. Canopy N content (kg N ha^{-1}) at GS 59 as the sole variable explained (P<0.001) 84.5% of variation in grain N concentration (Model 4a, Table 3.6). When GS 59 biomass (kg DM ha^{-1}) was included as a second variable it had a significant effect (P<0.001) and the level of variation accounted for increased to 91%. Including GS 59 soil mineral N content (kg N ha^{-1}) had a significant effect (P<0.013), but reduced the variation explained to 87%.

Table 3.7 shows Model 5 which used GS 59 N content (kg N ha^{-1}) as the first explanatory variable of grain N concentration adding the number of potential grain sites at GS 59 (P=0.002) increased the amount of variation accounted for to 87.1%. The addition of GS 59 soil mineral N content did not have a significant effect (P=0.655).

Model 6 (Table 3.8) shows the explanatory variables of grain N concentration from the two 2014 experimental sites. Canopy N content (kg N ha^{-1}) was with the combination of GS 59 biomass (kg DM ha^{-1}) and the number of potential grain sites (m^{-2}) at GS 59 as the explanatory variables. However, potential grain sites did not have a significant effect in the model and neither did the inclusion of GS 59 soil mineral N content (P<0.078).

3.4 **Discussion**

Varying fertiliser N supply at three experimental sites gave a large range in crop growth, grain yield and grain N concentration greater than the range found in commercial practice. Across the three site-seasons the mean grain yields achieved ranged from around 3 t ha^{-1} to 7.5 t ha^{-1} and from 1 to 2.5% grain N concentration. This has provided a data set for testing the main determinants of grain N concentration in spring barley and for investigating the scope for predicting N concentration from crop and soil measurements made at ear emergence.

An ideal grain N concentration prediction method would include as few explanatory variables as possible, which simultaneously account for a large amount of the variation in the response variable. The relationships between explanatory variables and the response variable would be robust across sites, years and management practices, but also such variables would be quick and easy for a producer to measure.

Grain N concentration is the quotient of grain yield (kg DM) and grain N content (kg N) per unit area. As neither grain N content nor grain yield on their own could account for a significant proportion of the variation in grain N concentration when all sites were combined, it can be concluded that both variables may need to be included in a prediction method of grain N concentration.

The relationships between grain N content and grain yield and the response variable grain N concentration showed differences between the two experimental years. At Road Field (2013), canopy growth was minimal between GS 39 and 59 and final canopy size was small suggesting that some restriction to growth occurred prior to flowering and grain filling. NNI and tissue N concentrations were high indicating that the small canopy produced was not the result N deficiency. An NNI greater than 1.0 indicates that luxury accumulation of N in the crop has occurred (Lemaire *et al*., 1989). Additionally, mineral N levels in the soil at GS 59 were high when compared to the other sites (Bull Park and College Field, 2014) suggesting poor uptake of N, possibly because some other factor such as water stress, limited crop demand. In 2013 rainfall was below the long term average for the months of April to July (inclusive) representing the majority of the growing season when canopy expansion

occurs, yield components are formed and early grain development takes place. Soil moisture deficits at ear emergence were calculated for 2013 and 2014 and the results indicate that plant available water was depleted up to 63% of field capacity in Road Field 2013 compared to 14 and 21% depletion of plant available water at Bull Park and College Field respectively in 2014. Thus soil moisture deficit data indicate the crop suffered water stress in 2013. It can be deduced, therefore, that the water stress reduced leaf area expansion (as described by Farooq *et al.*, 2009 and references within) and ultimately led to reduced solar radiation capture, decreased growth rate and biomass accumulation (Jamieson *et al*., 1995).

There was a strong positive relationship between grain N content and total aboveground crop N content at harvest (R^2= 0.96). The relationship was across a range of crop N contents, grain N contents, sites and seasons. McMullen *et al. (*1988) and Delogu *et al. (*1998) both found that total N content of the crop at maturity was highly correlated to grain N content. This relationship would be expected; grain N content (the kg N present in the grain) is the result of accumulated N present in the plant and the NHI (partitioning of N between grain and the remainder of the plant) (Cox *et al*., 1985) and NHI has been reported to be quite stable (McMullan *et al*., 1988; Wetselaar and Farquhar, 1980). Indeed, in the current study NHI did not explain much of the variation in grain N content as the relationship was not significant. The relationship between grain N content and NHI was quite comparable across treatments, sites and seasons with little change in NHI. Thus, total aboveground N content was associated with a greater level of variation in grain N content than NHI. With this information, NHI can be removed as a possible variable to be included in the prediction of grain N concentration.

The greatest quantity of N present in the grain at maturity is generally N remobilised from pre-anthesis N assimilation. Bulman and Smith (1994) concluded that although a plant's ability to accumulate N post heading contributed towards the determination of total plant N and grain N, its influence upon the partitioning of N within the plant at harvest or the proportion of N in the grain is minimal in barley. Conversely, post anthesis N uptake (PANU) can provide significant contributions to total crop N content (Austin *et al*., 1977; Perez *et al*., 1983; Masclaux *et al*., 2001; Van Sanford and MacKown, 1987; Bingham *et al*., 2011).

PANU can be increased through high levels of soil N at grain filling and can reduce the remobilisation and therefore importance of pre-anthesis N (Papakosta and Gagianas, 1991). However, in the current study there was no relationship between PANU and soil mineral N content at GS59. The soil mineral N content at Bull Park and College Field in 2014 was low at this growth stage over a wide range of fertiliser N rates, presumably because crop growth and N uptake prior to flowering had depleted the soil of fertiliser N. At Road Field in 2013, soil mineral N content at flowering was considerably greater, reflecting a smaller crop uptake at this site. Here, dry soil and a restricted crop demand may account for the low PANU in spite of appreciable quantities of available mineral N.

The relationship between total aboveground N content at harvest and GS 59 canopy N content was very strong, across the range of N treatments and N contents, sites and seasons. These results are comparable with findings in winter and spring barley and winter wheat that found that between 60 and 90% of total crop N present at maturity was contained within the plant at anthesis (Austin *et al*., 1977; Heitholt *et al*., 1990; de Ruiter and Brooking, 1994; Bulman and Smith, 1994). Determination of crop N content involves the destructive sampling of a known area of the crop, oven drying the material to a constant weight, grinding and subsequent N concentration analysis. This method is relatively time and labour intensive; however it can provide accurate estimations of canopy N content.

Yield of small grain cereal crops can be described as either the number of grains per unit area and mean grain weight or as the result of harvest aboveground biomass and harvest index. If the former method of yield explanation is examined, the relationship between grain yield and harvest grain number was quite robust, with a significant relationship produced explaining 77% of variation across the range of treatments, grain numbers, grain yields, sites and seasons. This would agree with the literature which investigated the relationship between grain yield and grain number across a range of environments (Abeledo *et al*., 2003; Bingham *et al*., 2007a; Gallagher *et al*., 1975: Serrago *et al*., 2013; de Ruiter and Brooking (1994); Cossani *et al*., 2009). As expected grain weight did not account for as large a proportion of variation in grain

yield as grain number, in agreement with previous findings (de Ruiter and Brooking, 1994; Bingham *et al*., 2007b), showing grain weight has less of an impact on grain yield than grain number in barley. This would suggest that predictors of grain number at harvest might be useful variables to include in the prediction of grain N concentration. Harvest grain number showed a modest relationship with potential grain sites at ear emergence. The calculation of potential grain sites at ear emergence requires the calculation of ears m^{-2} and potential grain sites per ear. This process is relatively quick and easy for producers to conduct and can be repeated at a number of sites in a field to achieve an accurate estimation.

Grain yield can also be described as the end product of final aboveground biomass and harvest index (HI). Grain yield produced a positive and significant ($P<0.001$; $R^2=0.83$) relationship with total harvest dry matter accumulation. Cossani *et al. (*2009) found that the total biomass at maturity followed the patterns observed for grain yield. In the current study, HI accounted for a low level of variation in grain yield which conflicts with the findings of Singh and Stoskopf (1971), but agrees with Peltonen-Sainio *et al. (*2008). Additionally, the resultsof this study are in agreement with those of White and Wilson (2006) who also found that variation in grain yield was strongly associated with variation in biomass rather than HI. The results of our relationships would indicate that predictors of harvest biomass would be a superior parameter to use in a prediction system for grain N concentration as HI is likely to be relatively stable unless a significant post-anthesis drought or disease restricts grain filling. Furthermore, a significant correlation between total dry matter at maturity and crop biomass at ear emergence was found. These results concur with those of Przulj and Momcilovic (2003) who found a strong relationship between both variables, who concluded that in a range of environmental conditions and N fertiliser rates, approximately 50% of total dry matter at maturity was present at anthesis. Biomass or dry matter accumulation is a relatively simple and easy variable to measure although it involves the oven drying of the sample to a constant weight.

Estimates of crop growth at ear emergence were evaluated for their relationship with grain yield and grain N content. As the literature and this research shows, grain N content is related to total N content at harvest which in turn is strongly related to GS 59 N content. Therefore, it is sensible to propose that GS 59 canopy N content and

grain N content are strongly related. Palta and Fillery (1995) found similar results in wheat for anthesis N and grain N content. Actual grain numbers at harvest and grain yield showed a significant relationship with potential grain site estimates at GS 59, but accounted for only a modest 52 and 31% of the variation respectively. Neither relationship was robust across, sites and seasons. Although the number of potential grain sites would be relatively easy for a producer to measure, they are prone to several sources of error. The number of potential sites was estimated from the number of spikelets per ear and the number of ears per m^{-2}. Unsuccessful fertilisation of some spikelets and late ears emerging after assessments at GS59 (Kennedy *et al*., 2016) could contribute to the unexplained variation in actual grain numbers observed here. An additional source of unexplained variation in the relationship between yield and potential grain sites is the variation in mean grain weight between site-seasons. Consequently, as a large proportion of the variation in yield remained unexplained, use of the number of potential grain sites in predictions of grain N concentration may not prove to be particularly accurate.

As grain yield is the product of harvest biomass and HI and that HI is known to be relatively stable across sites, it is sensible that a strong relationship exists between grain yield and biomass at maturity. The relationship between harvest biomass and crop biomass at flowering is also well documented (Dyson, 1977; Papakosta and Gagianas, 1991; Dordas, 2012). Therefore a strong relationship between grain yield and GS 59 biomass would be expected. This has been proven in both wheat (Turner, 1997) and barley (Ramos *et al*., 1985). In the current study the relationship between GS 59 biomass and grain yield accounted for almost 90% of variation across the three site-seasons and suggests that GS 59 biomass is a promising variable to be included in a prediction model.

On the basis of the relationships discussed above, the most promising variables to use to explain variation in grain N concentration were canopy N content and crop biomass at ear emergence. These were used in multiple regression models to determine how much of the variation in grain N concentration could be accounted for in data from all three site-seasons or just the 2014 sites. The number of potential grain sites and soil mineral N content were also investigated as possible explanatory variables, because there are plausible mechanisms to link them to yield and grain N

content. However, for the reasons discussed above they were not expected to contribute significantly to variation in grain N concentration.

When data from all three site-seasons were pooled, the model that explained the most variation was model 1c in which the explanatory variables were canopy N content, biomass and soil mineral N content. Given the lack of relationship observed between soil mineral N content and PANU and between PANU and crop N content at harvest, it was surprising that soil mineral N content accounted for any appreciable variation in grain N concentration. Rather than being functionally linked to grain N concentration, it is likely that soil mineral N correlated with another factor associated with variation in grain N concentration between site-seasons such as the soil moisture deficit. The soil moisture deficits at ear emergence were large in 2013 and accompanied by large soil mineral N contents. Low mean grain weights resulting from restrictions to grain storage capacity or grain filling by crop water stress would increase the grain N concentration in 2013, but not 2014 and thus correlate with the greater soil mineral N in 2013 compared to 2014.

Models in which the number of potential grain sites was substituted for crop biomass (Model 2) explained less of the variation in grain N concentration. This was expected given that the potential grain numbers was only weakly associated with grain yield.

When data from just 2014 were analysed, the best model (the one that accounted for the most variation in grain N concentration) was again the one that used canopy N content and crop biomass as explanatory variables (Model 4b). By contrast to the three site-season models, inclusion of soil mineral N content failed to explain any more of the variation. This is probably because there was no confounding effect of water stress in 2014 and relatively little variation in soil mineral N across treatments at Bull Park and College Field.

In conclusion, the results presented in this Chapter show that variation in grain N concentration observed across site-seasons and fertiliser N treatments was related to variation in both grain N content and yield. In turn, grain N content was strongly and positively related to canopy N content measured at ear emergence and yield to crop biomass at ear emergence. These relationships were reasonably robust across sites and seasons. Multiple regression models using ear emergence measurements of

canopy N content and biomass as explanatory variables accounted for between 80-91% of the variation in grain N concentration at harvest depending on the site-seasons included in the analysis. These statistical models could form the basis of a system to predict grain N concentration from crop measurements at ear emergence. The accuracy of predictions now needs to be tested using independent data (i.e. data not used in the development of the statistical models).

4 Validation of Model Predictions

4.1 Introduction

Grain nitrogen concentration is an important indicator of quality in cereal crops, such as winter wheat (*Triticum aestivum L.*) and spring barley (*Hordeum vulgare L.*). Grain N concentration requirements differ for different markets including malting 1.52-1.84% (Söderström *et al*., 2010) and distilling of less than 1.5% (Spink *et al*., 2014). Satisfying such requirements provides a price premium compared to the lower end markets such as animal feed, where moisture content and hectolitre weight are the main quality parameters (Spink *et al*., 2014). The ability to accurately and reliably forecast the final grain N concentration of malting barley early in season would afford advantages to both producer and industry. Prediction of grain N concentration would benefit growers regarding harvest management. Crops predicted to be marginally below the grain N concentration requirements for malting markets may have late season nitrogen fertiliser applied to correct nitrogen concentration levels (Gooding *et al*., 2007; Woolfolk *et al*., 2002; Wuest and Cassman, 1992), . Moreover, crops predicted to be physiologically or economically beyond correction or those above the maximum acceptable concentrations could be harvested last for other markets such as animal feed, thereby allowing growers to prioritise harvesting of the most valuable crops. Crops physiologically or economically beyond correction would include those whose grain N concentration is unlikely to be raised sufficiently by late season fertiliser-N applications for it to meet grain N specifications, or where the cost of the N required to do so would exceed the economic return from the additional premium for grain quality. Using prediction models to inform decisions about crop management on a field by field basis can be considered as the *tactical* use of grain N prediction. Maltsters have expressed a strong interest in using prediction models in a more *strategic* sense. A regional and national forecasting of grain quality before harvest would allow them to facilitate logistics of harvest produce based on geographical location and predicted regional grain N concentration and subsequent price planning.

At present there is no prediction model available commercially that can reliably forecast grain N concentration of barley. Producers use best practice recommendations to achieve the required grain quality such as choice of variety, sowing date, nitrogen rate and application timing and field history in conjunction with knowledge of previous farm grain N concentration achievements. At the moment, industry maltsters have no projection of the expected malting barley harvest in terms of either volume or quality until on-site analysis at intake.

In Chapter 3 statistical models were produced that were able to explain a large proportion of the variation in grain N concentration in N response experiments using a limited number of variables measured at ear emergence. These models may, therefore, form the basis of a forecasting system for grain N concentration. However, in order for growers and industry to have confidence in the forecasts, model predictions must be tested thoroughly across the range of environments, seasons and crop management regimes that the forecasting system is intended to be used in. Good correlations have frequently been reported between a range of crop characteristics and yield or grain N concentration (Pettersen and Eckersten, 2007; Weightman *et al*., 2011; Molina-Cano *et al*., 2001). However, where data have been taken from different experiments and sites, relationships between measured variables and grain N concentrations tend to be weaker, and the amount of unexplained variation in grain N concentration greater (Weightman *et al*., 2011). Few studies have tested these models widely across sites and years using completely independent data sets.

Weightman *et al. (*2011) investigated the use of tissue N concentrations in whole plant and ear samples as predictors of grain N concentration in winter wheat for bread making markets. Samples collected at the milky ripe stage produced better relationships with grain N concentration than those taken at anthesis, while ear samples performed as well as whole plants. However, regression models produced using trial data sets gave very poor predictions of grain N concentrations in independent measurements on commercial crops (R^2 = 0.19 for predicted versus observed) with a general bias towards under-prediction. As discussed in Chapter 3, grain N concentration is a quotient of grain N and grain yield per unit area. As such,

methods to predict grain N concentration from estimates of either variable individually are likely to underperform in terms of prediction accuracy. Factors that influence either grain N accumulation or starch deposition (yield formation) will both contribute to variation in grain N concentration between crops. For example, site factors such as soil texture, structure and organic matter content can affect grain N concentrations through effects on crop growth and soil N availability (Malik *et at*., 2012: Masoin *et al*., 2007 : Wang *et al*., 2012; Ros *et al*., 2011). In addition, variation in crop management can also influence grain N concentration, including sowing date (Subedi *et al*., 2006: Conry, 1984) seed rate (Edney *et al*., 2012: Gooding *et al*., 2002; Geleta *et al*., 2002), N levels (Birch and Long, 1990) and previous cropping (Zou *et al*., 2015; Hedlin *et al*., 1957).

The tactical and strategic value of any model used to forecast grain N concentration will, therefore, depend on the accuracy of its predictions across sites, seasons and common crop management practices. Validation data was collated from a number of sources:

I. The ability of the models to predict grain N concentrations of crops grown at non-standard seed rates was tested using data from lower and higher seed rates included in the N response experiments where the standard seed rate was used to develop the models in 2014.
II. Measurements were made in 2015 on an N response experiment repeated at two contrasting sites using the same variety (*SY Taberna*) as that employed in the development of the model in 2013 and 2014, to provide a test of the accuracy of predictions in a different season, but under a comparable crop management regime to that used in the development of the model.
III. Commercially grown crops were sampled in 2015 which included cultivar *SY Taberna* plus a number of different varieties, to provide a wider range of varieties and sites to test the models.

Collectively, these analyses sought to evaluate the robustness of the prediction models across soil types, variety, seed rate and season to determine their value as a possible forecasting method for use by producers and industry.

If grain N concentration is predicted to be unsatisfactorily low for malting market specifications, late season post-anthesis N fertiliser application may rectify the situation by increasing grain N concentration (Dampney and Salmon, 1990; Gooding *et al*., 2007) above the minimum acceptable threshold. Late season fertiliser nitrogen applied around anthesis to wheat in both foliar and granular forms were found to have fertiliser recovery efficiencies of between 17-21 and 15% in the grain (Gooding *et al*., 2007). However, in order to aid decisions about the application of late season N, growers will need guidance on the amount of N to be applied, the efficiency with which the fertiliser N is recovered by the crop and the scale of increase in grain N concentration that can be achieved.

The main objectives of experiments reported in this chapter were 1) to investigate the effect of seed rate on the crop's response to N fertiliser, 2) to investigate the effects of applications of fertiliser N at anthesis on N uptake and grain N concentrations in barley crops of different N status prior to grain filling and 3) to use independent data sets to test the ability of the models presented in Chapter 3 to predict grain N concentrations across a range of common management practices, sites and seasons.

4.2 Materials and Methods

4.2.1 Effects of seed rate on crop growth, yield and grain N concentration

Full details of seed rate treatments are given in Chapter 3.2, Tables 3.1 and 3.2. The experimental design was a randomised complete block with four replications. The treatments consisted of a range of fertiliser N rates and timings. In 2014, both low and standard (150 and 300 seeds m^{-2} respectively) were included at Bull Park and College Field and included N rates from 0 – 200 kg N ha^{-1}. An additional seed rate of 600 seed m^{-2} was included in the experiment at one level of fertiliser N application.

Experimental plots were sown as two 2.3 x 9.3m plots with one plot designated for destructive sampling throughout the growing season and the other for non-destructive measurements and combine harvesting for determination of yield, grain N and other quality characteristics.

Data from the seed rate treatments were analysed to determine the effects of these treatments on canopy growth, N content, post-anthesis N recovery and grain N concentration over a range of fertiliser N supplies. The data for the low seed rate treatment were then used to predict grain N concentrations using the multiple regression models developed from the standard seed rate and data presented in Chapter 3.3. This provides a test of the accuracy of predictions when seed rate is varied beyond the standard recommended value.

The data were analysed by Anova (Genstat, 13th Edition, VSN International Ltd.; Hemel Hempstead, UK) as a three-way factorial fully randomised block design with four replications; N Rate*Seed Rate*Site were the treatment factors and Site*Rep was used as a blocking structure. The results for the three levels of the seed rate treatment (150, 300 and 600 seeds m^{-2}) were analysed separately as a two factor randomised block design with Seed Rate*Site as the treatment factors.

All data were checked for a normal distribution of residuals and homogeneity of variance and no transformation was necessary. The least significant difference (LSD) was calculated at the 5% significance level. Least squares linear regression analysis was used to compare predicted values of grain N concentration to measured values

and the goodness of fit determined by calculation of the root mean square error (RMSE) as described below for experiment 2.

4.2.2 Effects of late fertiliser N application on grain N concentration

Full details of fertiliser N treatments at ear emergence are given in Chapter 3.2, Tables 3.1 and 3.2. The experimental design was a randomised complete block with four replications. The treatments consisted of a range of fertiliser N rates (0 – 200 kg N ha^{-1}) and timings. Late N application treatments were included in one site in 2013 and two sites in 2014, in addition to basal N treatments at standard seed rates. However, both basal N rates and late N application supplies were altered between years. All treatments were fully randomised within blocks. Experimental plots were sown as two 2.3 x 9.3m plots with one plot designated for destructive sampling throughout the growing season and the other for non-destructive measurements and combine harvesting for determination of yield, grain N and other quality characteristics.

Data from the late fertiliser N application were analysed to quantify the efficiency of recovery by the crop and its effects on grain N concentration.
In addition to the calculations outlined in Chapter 3, the apparent recovery of late fertiliser N application (ANR) was calculated using the equation:

$$ANR = (UF - U0) / NF$$

where U_F is the N content of the aboveground biomass when an amount N_F of fertiliser N is applied at flowering and U_0 is the uptake when no late fertiliser N is applied. Additionally, apparent fertiliser recovery by grain of N applied at flowering was calculated where, in this case, U_F is the uptake of N by the grain when an amount N_F of fertiliser N is applied and U_0 is the corresponding uptake by grain when no fertiliser is applied (Greenwood *et al*., 1989).

Harvest index (HI) was calculated by dividing total grain yield (GY, expressed at 100% dry matter content) by total aboveground biomass (AGB):

$$HI = \left(\frac{GY}{AGB}\right) * 100$$

Nitrogen harvest index (NHI) was calculated as given by Austin and Jones (1975):

$$NHI = \left(\frac{GY * grain\ N\ conc}{Straw\ biomass * straw\ N\ conc + GY * grain\ N\ conc}\right) * 100$$

where straw biomass is the dry weight of leaf, stem, leaf sheath and ear chaff.

All data were checked for a normal distribution of residuals and homogeneity of variance and no transformation was necessary. Because the basal N fertiliser regime differed between the years the 2013 and 2014 data were analysed separately. The 2014 data were analysed by Anova as a three factor randomised block with four replications with Basal N Rate*Late N*Site as the treatments. The 2013 data were analysed as a two factor design with Basal N Rate*Late N as the treatments. The least significant difference (LSD) was calculated at the 5% significance level.

4.2.3 Validation of predictions of grain N concentration across sites and seasons

Two sets of data were collected to provide an independent data set to test the accuracy of predictions of grain N concentration using the models presented in Chapter 3. The first comprised samples taken from two nitrogen response experiments which received fertiliser N application rates ranging from 0 to 240 kg ha^{-1} in different fields at Teagasc, Oak Park, Co. Carlow, Ireland in 2015. These were used to test the models on the same cultivar of barley, but grown in a different season and over a wide range of nitrogen nutritional status and yield.

Samples were also taken from 34 commercial crops of spring barley grown at various locations in the south east of Ireland representative of the malting barley production areas. These included crops of the same variety (SY Taberna) as that used in developing the prediction models, plus two other popular malting barley varieties (Propino and Sanette). All crops were managed according to recommended commercial agronomic practice. Crops were sown at industry recommended seed rates of between 250 and 350 seeds per m^{-2}. Fertiliser and pesticide applications were carried out according to requirement based on professional agronomic advice. Details of site location and husbandry are given in Appendix 3.

4.2.4 **Crop measurements**

At ear emergence, areas (3 x 10 m) of the commercial crops were identified and marked for sampling at physiological maturity (referred to here as the pre-harvest sample). Sample areas of experimental plots utilised the entire 2.3 x 9.3m. Plants were then sampled from the area adjacent to the designated pre-harvest sampling area to provide an ear emergence sample. A guard width of 0.5 m was left between the ear emergence and pre-harvest sampling areas to avoid the introduction of edge effects. At ear emergence, plants were removed by hand from 2 x 0.5m lengths of adjacent rows located on each of the four sides of the selected pre-harvest sample area within the commercial crops; these samples were bulked together to form two smaller sample sizes consisting of an A and B sample to reduce sampling error. This sampling regime was to ensure that ear emergence and pre-harvest samples were taken from the same part of the crop to minimise spatial effects on growth from variation in soil conditions and plant establishment. Samples of the same size were taken from sampling plots within the N response experiments at Oak Park and subsequently treated in the same way. Plants were transported to the laboratory where they were stored in the dark at $4^{o}C$ in sealed plastic bags, until processing and analysis. All processing was completed within 48 hours of sampling. For the ear emergence samples, roots were removed from the shoots at ground level and discarded. The shoot sample was weighed fresh and subdivided into ten

approximately equal subsamples. Two subsamples were selected at random, bulked together and weighed fresh before separating shoots into leaf, stem and ear fractions. The fractions were dried in a fan-assisted oven at 70 ^{o}C for at least 48h (or until constant weight) and weighed to the nearest gram for determination of biomass. The stem fraction was further separated into true stem and leaf sheath. Tissue was retained for determination of fraction N content. The number of potential grain sites (i.e. spikelets) per ear and ear length was recorded on twenty randomly selected ears from this sample post-drying. A third subsample was weighed and used for green area index (GAI) determination. The projected area of leaf laminae, stem (including leaf sheath) and ear were measured separately using a WD3 – WinDIAS Leaf Image Analysis System (Delta-T Devices Ltd, Cambridge, UK) and total GAI calculated. A further subsample was weighed and the number of shoots counted. This and the remaining six subsamples were then bulked together, weighed fresh and then oven dried for measurement of total aboveground biomass and crop N content. Potential grain numbers per ear were multiplied by shoot number per m^{-2} to calculate potential grain sites per m^{-2}. Values of biomass, shoot and grain numbers of samples were converted to area based measurements using the length of crop rows sampled and the measured inter-row width after first accounting for the subsampling by the ratio of sub-sample:whole sample fresh weight.

Pre-harvest samples were taken at physiological maturity from the areas marked at ear emergence. Samples (4 x 1 m length of row) were removed from within (3 x 10m) sample areas identified at ear emergence. Roots were removed from the shoots at ground level and the sample weighed; 20 shoots were taken at random, weighed and separated into leaf, stem and ear. Stems were additionally separated into true stem and leaf sheath. Ears were separated into portions of grain and chaff by hand to calculate the number of grains per ear and additionally biomass partitioning between grain and chaff and N content determination. All fractions were oven dried at 70^{o}C in a fan oven before determination of biomass (dry weight). The remaining pre-harvest biomass sample were threshed using a laboratory threshing machine (Wintersteiger AG, Austria) separating grain and remaining straw biomass for determination of both nitrogen content and harvest index. Grain was ground to <0.5mm using a sample mill (Foss Cyclotec 1093; Höganäs, Sweden), straw biomass was ground to <2.00mm in a

cutting mill (RetschMühle. Retsch GmbH Haan, Germany) and subsequently finely ground (<1mm) in a hammer mill (Polymix – PX-MFC 90D, Kinematica AG, Switzerland) prior to analysis for nitrogen content by means of the Dumas combustion method using a Rapid N Cube (Elementar Analysensysteme GmbH), using a sample size of approximately 400mg. Values of biomass, grain yield and N contents of samples were converted to area based measurements as described above for the ear emergence samples.

Soil was sampled at ear emergence to estimate available soil mineral N (nitrate and ammonium) content. Samples were collected after plant sampling with a 20mm diameter auger at 0-30 and 30-60 cm depths at four locations for each crop sampled and pooled together at each specific depth for analysis. Samples were maintained at 2-4°C using ice blocks and insulated boxes and stored within 2 hours by freezing (-20°C) to await mineral nitrogen extraction. Frozen soil samples were allowed to thaw for a period of 16 hours at 2-4°C in a refrigerator and extracted within 24 hours of the start of thaw. Frozen storage and a slow thaw (16 hours at 2-4°C) can result in a lower amount of nitrate N and a higher amount of ammonium N, but no overall affect upon total soil mineral nitrogen (Kindred *et al*., 2012).

De-stoned soil (100g) from which visible organic debris (roots and straw) had been removed by hand was shaken with 200ml of 2M KCl for 60 minutes on a rotational shaker (New Brunswick Scientific, Model G-10 Gyrotary; Edison, NJ, USA). The soil slurries were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK) and the extract was frozen for analysis at a later date. Ammonium and nitrate content was determined by colorimetric analysis on a Skalar San++ Automated Wet Chemistry Analyser also known as Continuous Flow Analyser (Skalar Analytical B.V, The Netherlands). Soil moisture content was measured gravimetrically by oven drying a subsample at 105°C for 24 hours. Bulk density of the soil was estimated according to Rowell (1994).

4.2.5 **Statistical analysis**

Simple linear least squares regression was used to analyse the relationship between actual grain N concentrations and those predicted from measurements at ear emergence using the various models presented in Chapter 3. Analysis was conducted in GenStat (13th Edition, VSN International Ltd.; Hemel Hempstead, UK). The goodness of fit between actual and predicted values was estimated using the root mean square error (RMSE) (Makowski, 1999) and the slope of the regression. The RMSE measures the difference between values predicted by a model and the values actually observed from the environment that is being modelled.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (Observed - Predicted)^2}{n}}$$

Both confidence and prediction intervals describe the variability of a range of values that indicate, with 95% certainty for example, that a repeat of the observations will occur within the interval for a given prediction value. A regression equation describes the population of the relationship examined. The larger the sample size, the greater the likelihood that the fit will be close to the "true" relationship. The confidence interval presents the range of likely results that may be achieved if the process is repeated. So the confidence interval summarises the uncertainty in the slope and intercept of the regression line and describes how well the regression works in providing the population parameters (the true value in this case).

The use of a fitted line for prediction gives only one estimate of the response for a given explanatory value and this value can be calculated from the fitted parameters (intercept and slope) or read off the line on a plot.

The confidence interval describes the spread of means of the same number of observations that produced the regression line. Due to the large number of observations in each mean, while showing variability, they are generally close to the

true mean. Hence the distribution of means is narrower than the distribution of individual observations. However, prediction involves estimating an outcome for an individual observation, and not for a mean as above. It is clear that such an estimate cannot be substantially less variable than the actual spread of the data since the spread indicates how far any individual can be from the fitted line. The prediction interval reflects the variability of individuals.

4.3 Results

4.3.1 Effects of seed rate on response of crop growth and yield to N fertiliser

The effect of lowering the seed rate on the crop's response to N fertiliser was measured at two sites. The main objectives were to quantify the N*seed rate interaction and to provide a set of data at lower than standard seed rate for testing predictions of grain N concentration. Thus, for simplicity of presentation, only the N*seed rate interaction means and main effects of seed rate and site are shown (Tables 4.1 and 4.2). Lowering the seed rate reduced aboveground biomass at ear emergence, when averaged across sites and N rates, the biomass was 6% smaller at the low seed rate (150 seeds m^{-2}) compared to the standard (300 seeds m^{-2}) ($P<0.001$; Table 4.1). However, seed rate did not alter the effects of N fertiliser on crop biomass (N*SR interaction $P=0.715$), nor was there a significant site*N rate*seed rate interaction. Averaged across sites and N rates, there was a small (1%) increase in green area index when seed rate was reduced from the standard seed rate (300 seeds m^{-2}) to the low rate (150 seeds m^{-2}) ($P=0.04$; Table 4.1). The effect of reducing seed rate did not impact the response of GAI to fertiliser N rate (N*SR interaction $P=0.685$), however, there was a significant site*N rate*seed rate interaction ($P=0.003$).

Altering the seed rates from a standard rate to a low rate reduced potential grain site numbers by approximately 1000 m^{-2} or 5% ($P=0.003$; Table 4.1). Lowering the seed rate did not affect the response of potential grain site number to fertiliser N rate ($P=0.818$), increasing N fertiliser application increased potential grain sites to a similar extent at both seed rates. Additionally, there was no site*N rate*seed rate interaction. Both seed rate and N rate had a significant effect on crop N concentration ($P<0.001$; Table 4.1) but the interaction between the two was not significant indicating that reducing the seed rate increased crop N concentration to a comparable extent (5-13%) at each N fertiliser application. As the increase in tissue N concentration at low seed rate was greater than the decrease in biomass, the crop N

content was increased by low seed rate when averaged over N fertiliser rates (P=0.012). However a significant interaction between N rate and seed rate indicated that the increase in crop N content was not uniform across N rates. The increase was greatest at the highest fertiliser supply (N*SR; P=0.034). Soil mineral N content were reduced by almost 25% when averaged across sites and N rates as a result of lowering the seed rate (P<0.001). A significant SR x N rate interaction indicated that the effect of seed rate on soil mineral N content was dependent on the rate of fertiliser N applied. At low seed rate, soil mineral N content were not affected by N fertiliser applications until the application exceeded 120 kg N ha^{-1}. By contrast at high seed rate the residual soil mineral N increased steadily with N application (N*SR; P<0.001).

When averaged across sites and N rates, grain yield was reduced by approximately 0.3 t DM ha^{-1} (P<0.001) by lowering the seed rate (Table 4.2). There was a significant interaction between N rate and seed rate (P=0.001) with reductions being greater (>0.6 t ha^{-1}) at N fertiliser rates of 160 kg N ha^{-1} or more. These differences in yield were associated with effects on grain number and mean grain weight. Reducing the seed rate reduced harvest grain number by 7% (P<0.001) and mean grain weight, was also significantly (P=0.005) reduced (2%) by low seed rate (Table 4.2). The largest mean grain weights (MGWs) were observed at the mid-range of N supply (80-120 kg N ha^{-1}). The effect of fertiliser N rate on harvest grain number was significantly altered (P=0.014) by lowering the seed rate, however the response of mean grain weight to fertiliser N rate was unaffected by the change in seed rate (N*SR, P>0.05). Additionally, there was no site*N rate*seed rate interaction for either component of grain yield. Lowering the seed rate increased grain N concentration by almost 7.5% when averaged across sites and N rates, from a mean value of 1.35% to 1.43% (P<0.001). The N rate*seed rate and site*N rate*seed rate interactions were not significant (Table 4.2). There was no effect of seed rate on grain N content when values were averaged across sites and N rates. At low application rates of N fertiliser, grain N contents tended to be greater at low seed rate than the standard, whereas the reverse was true at high N rates (N*SR; P=0.052).

Table 4.1. Effects of nitrogen fertiliser application (kg N ha^{-1}), seed rate (150 and 300 seeds m^{-2}) and site on crop growth characteristics at anthesis at two sites in 2014. Values are main effect (site and seed rate) or interaction (N x seed rate) means from a three factor ANOVA.

Factor		Biomass (t DM ha^{-1})		GAI		Potential Grain Sites (m^{-2})		N Concentration (%DM)		N Content (kg N ha^{-1})		Soil N Reserves (kg N ha^{-1})	
		Bull	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
Site		8.53	7.95	4.72	4.8	17130	19717	1.23	1.22	110.44	102.57	32.79	22.85
Seed Rate		**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**
		8.00	8.48	4.84	4.68	17946	18901	1.28	1.17	108.55	104.45	24.78	30.86
N Rate* Seed Rate		**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**
0		4.01	4.65	1.51	1.46	9615	10739	0.80	0.76	31.95	35.52	23.26	25.8
40		6.92	7.55	3.03	3.10	16811	17438	0.93	0.83	64.55	62.92	21.29	26.84
80		8.59	9.14	4.90	4.69	18914	20434	1.10	0.97	94.82	88.91	22.86	28.44
120		9.19	9.78	6.00	5.84	20644	21034	1.40	1.31	128.37	128.42	22.28	31.29
160		9.69	10.05	6.64	6.31	20434	21937	1.59	1.47	153.68	147.2	27.59	32.93
200		9.61	9.74	6.97	6.69	21258	21823	1.85	1.69	177.95	163.73	31.37	39.86
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	1	0.005	0.332	0.431	0.243	<0.001	948.8	0.648	ns	0.008	4.951	<0.001	1.69
Seed Rate (SR)	1	<0.001	0.217	0.04	0.151	0.003	607.3	<0.001	0.025	0.012	3.156	<0.001	0.876
N Rate (N)	5	<0.001	0.377	<0.001	0.262	<0.001	1051.9	<0.001	0.043	<0.001	5.467	<0.001	1.517
S*SR	1	0.421	ns	0.005	0.267	0.11	ns	0.089	ns	0.036	5.472	<0.001	1.789
S*N	5	0.092	ns	<0.001	0.394	0.432	ns	0.13	ns	0.076	ns	<0.001	2.425
N*SR	5	0.715	ns	0.685	ns	0.818	ns	0.078	ns	0.034	7.731	<0.001	2.146
S*N*SR	5	0.157	ns	0.003	0.538	0.087	ns	0.13	Ns	0.313	ns	0.242	ns
Residual df (Site)	6												

Table 4.2. Effects of nitrogen fertiliser application (kg N ha^{-1}), seed rate and site on final grain characteristics at harvest. Values are main effect (site and seed rate) or interaction (N x seed rate) means from a three factor ANOVA.

Factor		Grain Yield (t DM ha^{-1})		Harvest Grain Number (m^{-2})		Mean Grain Weight (mg at 100% DM)		Grain N Concentration (% DM)		Grain N Content (kg N ha^{-1})	
		Bull	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
Site		6.16	5.17	13906	12646	44.23	41.01	1.46	1.33	92.21	70.48
Seed Rate		**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**
		5.52	5.81	12817	13736	42.18	43.06	1.43	1.35	81.45	81.24
N Rate* Seed Rate		**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**	**Low**	**Standard**
0		3.48	3.19	8149	7818	42.67	40.80	1.18	1.10	41.29	35.28
40		4.77	4.99	10986	11812	43.38	42.20	1.19	1.14	57.37	57.34
80		5.90	6.11	13346	14078	44.23	43.38	1.29	1.17	76.87	72.06
120		6.39	6.67	14728	15488	43.39	43.04	1.49	1.44	95.89	96.41
160		6.26	7.00	14353	16485	43.62	42.34	1.65	1.54	103.87	108.18
200		6.29	6.91	15337	16732	41.09	41.32	1.80	1.71	113.38	118.15
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	1	<0.001	0.322	<0.001	377.9	<0.001	0.602	0.005	0.076	0.001	9.097
Seed Rate (SR)	1	<0.001	0.136	<0.001	377.9	0.005	0.602	<0.001	0.027	0.862	ns
N Rate (N)	5	<0.001	0.236	<0.001	654.5	<0.001	1.043	<0.001	0.047	<0.001	4.152
S*SR	1	0.116	ns	0.221	ns	0.914	Ns	0.019	0.077	0.78	ns
S*N	5	0.007	0.413	0.007	925.6	<0.001	1.476	0.251	ns	<0.001	9.866
N*SR	5	0.001	0.334	0.014	925.6	0.415	ns	0.617	ns	0.052	ns
S*N*SR	5	0.446	ns	0.693	ns	0.470	ns	0.437	ns	0.609	ns
Residual df (Site)	6										

4.3.2 Effect of low, standard and high seed rates at a single fertiliser N supply on grain yield, grain N concentration and N content

In a comparison of crops grown at high seed rate (600 seeds m^{-2}) with those at standard and low seed rate, there was a significant effect ($P<0.001$) of both site and seed rate on grain yield (Table 4.3). Grain yields averaged across seed rates were 20% greater at Bull Park than College Field. However there was no site*seed rate interaction. Increasing seed rate from low to standard increased grain yield by approximately 4%, while increasing the seeding rate further from low to high, resulted in a yield increase of 12%. Harvest grain number was affected by both seed rate and site ($P<0.001$), but there was no site*seed rate interaction ($P=0.840$). Altering seed rate from low to standard produced 5% more grain numbers m^{-2}, while grain number was 9% greater compared to the standard rate when high seed rates were used. Mean grain weight was significantly affected by site ($P=0.008$), but unaffected by seed rate and there was no site*seed rate interaction. Grain N concentration was affected by both site ($P=0.011$) and seed rate ($P<0.001$). When grain N concentration was averaged across seed rates, it was significantly higher in the Bull Park (1.52%) than in the College Field (1.33 %) ($P=0.011$). Grain N concentration was also significantly higher at lower than higher seed rates at both sites ($P<0.001$). Grain N content was significantly higher in the Bull Park than in the College field ($P<0.001$) but neither seed rate nor the site*seed rate interactions were significant.

Table 4.3. Effect of low (150 seeds m^{-2}), standard (300 seeds m^{-2}) and high (600 seeds m^{-2}) seed rates on grain yield and grain N concentration of spring barley grown at a fertiliser N application rate of 120 kg N ha^{-1} at Bull Park and College Field in 2014. Values are site means and mean for the site x seed rate interaction with four replicates.

Factor		Grain Yield (t DM ha^{-1})		Harvest Grain Number (m^{-2})		Mean Grain Weight (mg at 100% DM)		Grain N Concentration (% DM)		Grain N Content (kg N ha^{-1})	
		Bull	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
Site		7.34	6.15	16680	14672	44.11	41.97	1.52	1.33	111.30	81.80
Seed Rate											
Low		6.97	5.82	15580	13877	44.71	42.07	1.58	1.40	110.29	81.50
Standard		7.23	6.11	16564	14412	43.69	42.39	1.54	1.34	111.23	81.59
High		7.83	6.52	17894	15727	43.94	41.44	1.43	1.26	112.39	82.33
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	2	<0.001	0.236	<.001	763.2	0.008	1.500	0.011	0.125	<0.001	11.28
Seed Rate (SR)	2	<0.001	0.233	<.001	934.8	0.724	ns	<0.001	0.056	0.871	ns
S*SR	2	0.64	ns	0.840	ns	0.703	ns	0.83	ns	0.974	ns
Residual df (Site)	6										

4.3.3 Effect of fertiliser N application at anthesis on grain yield and components at differing basal nitrogen rates

In 2014 anthesis N fertiliser application resulted in a small (6%) increase (P<0.001) in grain yield when averaged over basal N rates (Table 4.4). The effects were greatest at zero and low basal N rates, and declined as the N rate was increased (N*aN interaction; P=0.038). Harvest grain number was unaffected by anthesis N application at any basal N supply. The effects of anthesis N on grain yield were associated with an increase in mean grain weight. The application of anthesis N fertiliser increased mean grain weight from 42.18 to 44.51 mg (P<0.001) when averaged over basal N rates and sites. As with grain yield, the effects tended to be greatest at zero and low basal N rates and decline as the N rate increased, however the interaction wasn't significant (P=0.124). In contrast at the Road Field site in 2013, anthesis N application did not alter the grain yield (P=0.807) or mean grain weight (P=0.653) (Table 4.6). Nor did it modify the response of grain yield and mean grain weight to N rate (N rate*anthesis N; P=0.787 and 0.311 respectively). However, anthesis N did increase grain number (P=0.004) a little (1%) although there was no N rate*anthesis N application interaction (P=0.621).

4.3.4 Effect of fertiliser N application at anthesis on grain N concentration, grain N content and N partitioning at differing basal nitrogen rates

The application of N fertiliser at anthesis increased (P<0.001) grain N concentration in 2014 and 2013 (Table 4.5 and 4.7). When averaged across sites and N rate in 2014, there was a 20% increase in grain N concentration from 1.35 % N to 1.63 % N (Table 4.5). Site, anthesis N, and basal N rate all had significant effects on grain N concentration (P<0.001). The interactions N rate*anthesis N application and site*N rate*anthesis N application on grain N concentration were also significant (P<0.001 and P=0.039 respectively). When averaged over sites, fertiliser N application at anthesis increased grain N concentration more in crops grown with low basal supplies compared to high. Grain N content was increased (P<0.001) by an average

of 25% or 20 kg N ha^{-1} by anthesis N application when mean values were calculated across site and basal N rate treatments in 2014. There was an interaction between basal N rate and anthesis N application (P=0.002) as the magnitude of the effects of anthesis N application on grain N content declined with increasing basal N supply (Table 4.5). Not only did anthesis N application increase grain N content, it also increased the straw plus chaff N content (P<0.001). The scale of the effect was again dependent on the basal N rate (N*aN; P=0.003), but here the increase following anthesis N application was greatest at high basal N supplies (Table 4.5). As anthesis N application increased both grain N content and the N content of straw and chaff, the nitrogen harvest index (NHI) was unchanged when averaged over sites and basal N supply (Table 4.5). However, there was a small increase in NHI with anthesis N at low basal N rates and a decrease at high basal rates giving an interaction between basal N supply and anthesis N (P=0.002). NHI declined with increasing basal N supply both with and without anthesis N application indicating a greater residual N in the straw and chaff and a reduction in relative partitioning to the grain.

Grain N concentrations at Road Field in 2013 were high (>2.0 % N when averaged over basal N rates (Table 4.7). The application of 30 kg N ha^{-1} increased the N concentration still further from 2.09 % to 2.26 % (P<0.001). Basal N rate also had a significant overall effect on grain N concentration (P<0.001), however there was no basal N rate*anthesis N interaction (P=0.569). The application of anthesis N had a large effect on grain N content, increasing it from 88.36 kg N ha^{-1} to 95.02 kg N ha^{-1} (P<0.001), but had no effect on the N content of straw and chaff or on NHI.

Table 4.4. Effect of fertiliser N application (40 k N ha^{-1}) at anthesis on grain yield and yield components Bull Park and College Field sites in 2014 following different basal nitrogen rates (kg N ha^{-1} applied during vegetative growth of the crop). Values are means for main effects of site, anthesis N treatment and for the basal N x anthesis N interaction.

Factor		Grain Yield (t DM ha^{-1})		Harvest Grain Number (m^{-2})		Mean Grain Weight (mg at 100% DM)	
		Bull	**College**	**Bull**	**College**	**Bull**	**College**
Site		6.57	5.38	14547	13096	45.41	41.28
Anthesis Nitrogen (aN)		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
		6.14	5.81	13907	13736	44.51	42.18
Basal N Rate*Anthesis N		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
0		3.80	3.19	8371	7818	45.53	40.80
40		5.44	4.99	12186	11812	44.61	42.20
80		6.59	6.11	13964	14078	48.29	43.38
120		6.92	6.67	15543	15488	44.49	43.04
160		7.03	7.00	16715	16485	42.01	42.34
200		7.03	6.91	16665	16732	42.14	41.32
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	1	<0.001	0.230	<.001	425.1	<0.001	1.291
Anthesis Nitrogen (aN)	1	<0.001	0.115	0.423	ns	<0.001	1.291
Basal N Rate (N)	5	<0.001	0.200	<.001	736.3	0.010	2.236
S*aN	1	0.106	ns	0.837	ns	0.152	ns
S*N	5	<0.001	0.323	0.016	1041.2	0.164	ns
N*aN	5	0.038	0.283	0.938	ns	0.124	ns
S*N*aN	5	0.655	ns	0.445	ns	0.732	ns
Residual df (Site)	6						

Table 4.5. Effect of fertiliser N application (40 k N ha^{-1}) at anthesis on grain N concentration, grain N content and N partitioning at Bull Park and College Field sites in 2014 at different basal nitrogen rates (kg N ha^{-1} applied during vegetative growth of the crop). Values are means for main effects of site, anthesis N treatment and for the basal N x anthesis N interaction.

Factor		Grain N Concentration (%)		Grain N Content (kg N ha^{-1})		Straw plus Chaff N Content (kg N ha^{-1})		Nitrogen Harvest Index	
		Bull	**College**	**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
Site		1.53	1.45	102.7	79.85	47.12	40.62	0.69	0.68
Anthesis Nitrogen (aN)		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
		1.63	1.35	101.31	81.24	50.62	37.12	0.68	0.69
Basal N Rate*aN		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
0		1.52	1.10	57.52	35.28	17.28	14.77	0.77	0.71
40		1.44	1.14	78.71	57.34	31.87	24.12	0.71	0.71
80		1.54	1.17	101.82	72.06	47.71	35.55	0.69	0.67
120		1.64	1.43	113.52	96.41	58.66	41.45	0.66	0.70
160		1.76	1.54	124.1	108.18	69.76	52.37	0.64	0.67
200		1.88	1.71	132.2	118.15	78.45	54.49	0.63	0.68
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	1	<0.001	0.031	<0.001	5.5	0.186	ns	0.762	ns
Anthesis Nitrogen (aN)	1	<0.001	0.022	<0.001	2.206	<0.001	3.087	0.428	ns
Basal N Rate (N)	5	<0.001	0.038	<0.001	3.821	<0.001	5.348	<0.001	0.028
S*aN	1	0.018	0.035	0.188	ns	0.155	ns	0.375	ns
S*N	5	0.418	ns	<0.001	6.868	0.005	11.81	<0.001	0.061
N*aN	5	<0.001	0.053	0.002	5.404	0.003	7.563	0.002	0.041
S*N*aN	5	0.039	0.076	0.6	ns	0.442	ns	0.52	ns
Residual df (Site)	6								

Table 4.6. Effect of fertiliser N application (30 k N ha^{-1}) at anthesis on grain yield and yield components at Road Field in 2013 at different basal nitrogen rates (kg N ha^{-1} applied during vegetative growth of the crop). Values are means for main effects of anthesis N treatment and for the basal N x anthesis N interaction.

Factor		Grain Yield (t DM ha^{-1})		Harvest Grain Number (m^{-2})		Mean Grain Weight (mg at 100% DM)	
Anthesis Nitrogen		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
		4.12	4.14	11146	11041	37.34	37.58
N Rate*Anthesis N		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
0		3.02	3.01	7700	7799	39.26	38.54
90		4.40	4.29	10956	11240	40.22	38.28
120		4.21	4.35	11473	11800	37.33	37.11
150		4.31	4.18	12240	11179	35.30	37.36
180		4.41	4.54	12240	12031	36.18	37.70
210		4.36	4.46	12269	12197	35.71	36.51
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Anthesis Nitrogen (aN)	1	0.807	ns	0.004	503.6	0.653	ns
Basal N Rate (N)	5	<0.001	0.2562	<0.674	ns	0.007	1.927
N*aN	5	0.787	ns	0.621	ns	0.311	ns

Table 4.7. Effect of fertiliser N application (30 k N ha^{-1}) at anthesis on grain N concentration, grain N content and N partitioning at Road Field in 2013 at different basal nitrogen rates (kg N ha^{-1} applied during vegetative growth of the crop). Values are means for main effects of anthesis N treatment and for the basal N x anthesis N interaction.

Factor		Grain N Concentration (%)		Grain N Content (kg N ha^{-1})		Straw plus Chaff N Content (kg N ha^{-1})		Nitrogen Harvest Index	
Anthesis Nitrogen		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
		2.26	2.09	95.02	88.36	29.47	29.69	0.76	0.75
BasalN Rate*Antheis N		**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**	**Anthesis N**	**No Anthesis N**
0		1.58	1.34	47.48	40.29	16.04	13.84	0.75	0.74
90		2.03	1.82	89.29	78.19	24.13	25.1	0.79	0.76
120		2.32	2.12	99.42	92.27	28.16	29.53	0.78	0.76
150		2.40	2.29	103.09	95.65	33.01	33.95	0.76	0.74
180		2.56	2.44	113.85	110.52	33.09	35.6	0.77	0.76
210		2.69	2.55	116.98	113.26	42.42	40.13	0.73	0.74
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Anthesis Nitrogen (aN)	1	<0.001	0.047	<0.001	3.489	0.87	ns	0.087	ns
Basal N Rate (N)	5	<0.001	0.0815	<0.001	6.043	<0.001	4.7	0.112	ns
N*aN	5	0.569	ns	0.803	ns	0.865	ns	0.834	ns

4.3.5 Apparent fertiliser recovery in grain and aboveground biomass from anthesis N at a range of basal N rates

The apparent recovery of anthesis applied fertiliser N in either above ground biomass or in grain did not differ between sites in 2014 (P=0.118 and P=0.087, respectively; Table 4.8). Recoveries were high with 0.75 to 0.93 of the N applied at anthesis being captured in the above ground biomass and 0.47 to 0.54 in the grain. The basal N fertiliser supply had no overall effect on the recovery of anthesis N by aboveground biomass (P=0.093), but did influence recovery by grain (P=0.001). Thus, at each site the greatest recovery by grain was observed at the intermediate basal N rate of 80 kg N ha^{-1}. At lower and higher basal N rates there was a general decrease in the apparent recovery of anthesis N by grain. A comparable relationship was witnessed at Road Field 2013 with the greatest recovery being found at a basal rate of 90 kg N ha^{-1} (Table 4.9), although recovery values were much lower than those of either site in 2014 averaging 0.22 for grain and 0.21 for above ground biomass.

Table 4.8. Apparent fertiliser recovery (increase in N content (kg)/kg fertiliser N applied) in grain and above ground biomass from 40 kg N ha^{-1} applied at anthesis to crops grown at a range of basal N rates (kg N ha^{-1} applied during vegetative growth of the crop). Sites were Bull Park and College Field 2014. Values are means of four replicate plots.

Factor		Grain N Recovery		Biomass N Recovery	
Site		**Bull**	**College**	**Bull**	**College**
		0.54	0.47	0.93	0.75
Basal N Rate		**Bull**	**College**	**Bull**	**College**
0		0.59	0.53	0.65	0.58
40		0.65	0.42	0.86	0.59
80		0.78	0.71	1.30	0.80
120		0.4	0.46	0.77	0.95
160		0.38	0.42	0.90	0.77
200		0.44	0.26	1.11	0.79
	df	**P**	**LSD**	**P**	**LSD**
Site (S)	1	0.087	ns	0.118	ns
Basal N Rate (N)	5	0.001	0.175	0.093	ns
S*N	5	0.484	ns	0.313	ns
Residual df (Site)	6				

Table 4.9. Apparent fertiliser recovery (increase in N content (kg)/kg fertiliser N applied) in grain and above ground biomass from 30 kg N ha^{-1} applied at anthesis to crops grown at a range of basal N rates (kg N ha^{-1} applied during vegetative growth of the crop) at Road Field 2013. Values are means of four replicate plots.

Factor		Grain N Recovery		Biomass N Recovery	
Basal N Rate					
0		0.18		0.23	
90		0.14		0.39	
120		0.18		0.15	
150		0.25		0.16	
180		0.11		0.11	
210		0.12		0.20	
	df	**P**	**LSD**	**P**	**LSD**
Basal N Rate	5	0.028	0.225	0.283	ns

4.3.6 Effects of different rates of fertiliser N applications at anthesis on grain N concentration

There was a significant difference in overall grain yield between the Bull Park and College Field of up to 23% ($P<0.001$) (Table 4.10). However there was no significant effect of anthesis N rate on grain yield averaged across sites ($P=0.185$) nor was there an interaction between site and anthesis N rate ($P=0.212$). Grain N concentration was higher in the Bull Park than the College Field ($P=0.024$) and was heavily influenced by fertiliser N rate at anthesis, increasing from 1.54% to 1.92% and 1.34% to 1.89% at Bull Park and College Field respectively as N applications increased from 0 to 120 kg N ha^{-1} ($P<0.001$). The site*anthesis N application interaction was also significant ($P=0.035$) as grain N concentration was increased to a greater extent at College Field (1.34 to 1.89 % N) compared to Bull Park (1.54 to 1.92 % N). Site and anthesis N rate both had highly significant effects on grain N content ($P<0.001$). Grain N content was 30 kg N ha^{-1} greater at Bull Park compared to College Field when averaged over anthesis application rates. At both sites, grain N content increased with each application of anthesis N, but with declining recovery (data not shown). There was no interaction between site and anthesis N content ($P=0.873$).

Table 4.10. Effect of varying fertiliser N application rates at anthesis on yield, grain N and N partitioning. Anthesis N was applied to crops grown with a basal fertiliser rate of 120 kg N ha^{-1} at Bull Park and College Field 2014.

Factor		Grain Yield (t DM ha^{-1})		Grain N Concentration (%)		Grain N Content (kg N ha^{-1})	
Site		**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
		7.54	6.11	1.74	1.66	131.50	101.60
Anthesis N		**Bull**	**College**	**Bull**	**College**	**Bull**	**College**
0		7.23	6.11	1.54	1.34	111.23	81.59
40		7.69	6.15	1.65	1.63	127.06	99.98
80		7.61	6.11	1.86	1.80	141.29	110.04
120		7.63	6.06	1.92	1.89	146.57	114.85
	df	**P**	**LSD**	**P**	**LSD**	**P**	**LSD**
Site (S)	1	<0.001	0.192	0.024	0.064	<0.001	5.86
Anthesis N (aN)	3	0.185	ns	<0.001	0.066	<0.001	6.45
S*aN	3	0.212	ns	0.035	0.096	0.873	ns
Residual df (Site)	6						

4.3.7 **Testing the accuracy of predictions of grain N concentration**

The grain nitrogen concentration prediction models developed in Chapter 3 which are tested in this section are summarised below. The explanatory variables; total crop N content, total crop biomass, counted number of potential grain sites, and soil mineral N content were all measured at GS 59.

1b = 1.9251+ 0.014177 * (N Content) - 0.0002335 * (Biomass)

1c = 1.427 + 0.00920 * (N Content) - 0.0001461 * (Biomass) + 0.00842 * (Soil Mineral N Content)

2b= 1.035 + 0.00672 * (N Content) - 0.0000073 * (Potential Grain Sites)

2c = 0.669 + 0.002337 * (N Content) - 0.00000354 * (Potential Grain Sites) + 0.01523 * (Soil Mineral N Content)

3b = 1.9251+ 0.014177 * (N Content) - 0.0002335 * (Biomass)

3c = 1.760 + 0.013212.* (N Content) - 0.0002403 * (Biomass) - 0.00001684 * (Potential Grain Sites)

4b = 1.1422 + 0.007120 * (N Content) - 0.0000631 * (Biomass)

4c = 1.0494 + 0.006589 * (N Content) - 0.0000608 * (Biomass) + 0.00415 * (Soil Mineral N Content)

5b = 1.0050 + 0.005610 * (N Content) - 0.00001273 * (Potential Grain Sites)

5c = 0.962 + 0.005373 * (N Content) - 0.00001117 * (Potential Grain Sites) - 0.00123 * (Soil Mineral N Content)

6b = 1.1422 + 0.007120 * (N Content) - 0.0000631 * (Biomass)

6c = 1.1787 + 0.007242 * (N Content) - 0.0000553 * (Biomass) - 0.00000613 * (Potential Grain Sites)

4.3.7.1 Accuracy across seed rates

The ability of models of grain N concentration developed using data from the standard seed rate (300 seeds m^{-2}) to predict the grain N concentration at a lower seed rate (150 seeds m^{-2}) is illustrated in Figures 4.1 and 4.2. Figure 4.1, shows a strong relationship of actual to predicted grain N concentration with a high level of variance accounted for (R^2=0.75) and a small RMSE (0.251). The predictions were made using model 1c developed from data from three site-seasons (Chapter 3.3). However, the slope of the line (0.856) is not parallel with the 1:1 reference slope (upper and lower 95% confidence limits = 0.99 and 0.72), indicating the data was not compatible with the line of perfect agreement. Figure 4.2, displays actual versus predicted grain N concentration when predictions were made using model 4c based on measurements of canopy N content, biomass and soil N reserves at ear emergence made over two sites in one year. This model provided a more accurate prediction in which the slope of the line was not different to 1.0 (upper and lower 95% confidence limits = 1.09 and 0.83), a greater level of variation in actual grain N concentration was accounted for (R^2=0.80) and the RMSE was smaller (0.114).

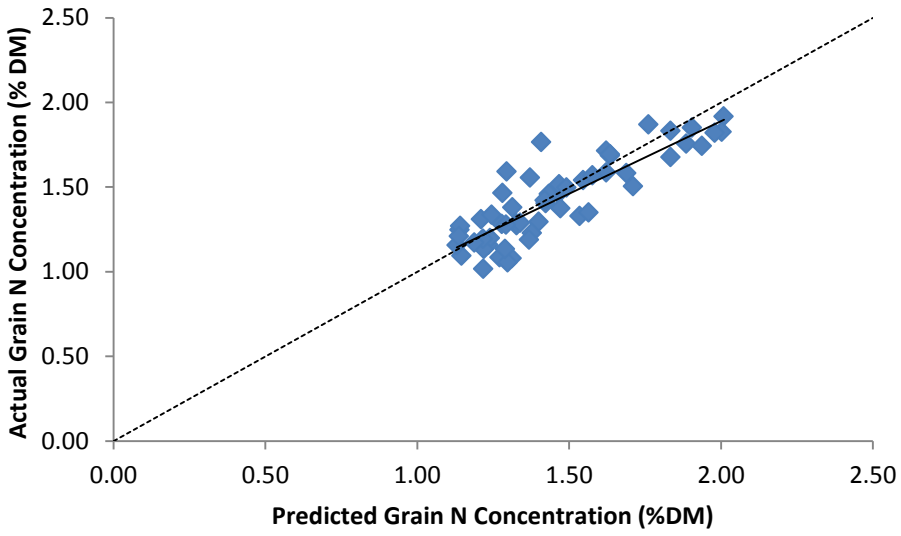

Figure 4.1. Actual versus predicted values of grain N concentration for crops grown at low seed rate (150 seeds m^{-2}). Values are for individual plots across N fertiliser treatments at Bull Park and College Field 2014. Predictions of grain N concentration were made using model 1 (c) developed from three sites across two years, using canopy N content, biomass and soil mineral N content at ear emergence as explanatory variables. RMSE = 0.251. 1:1 line is shown for reference. Slope of the line: y = 0.856x + 0.177; R^2 = 0.75.

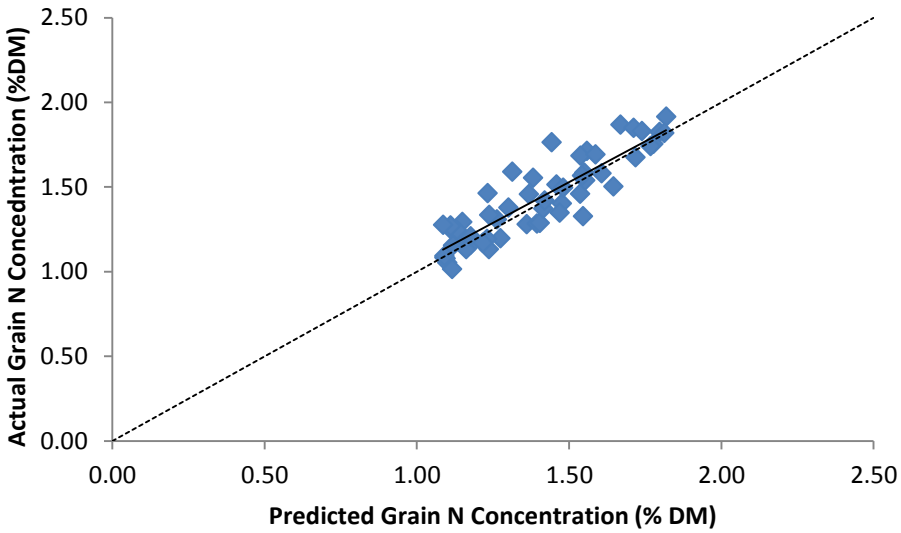

Figure 4.2. Actual versus predicted values of grain N concentration for crops grown at low seed rate (150 seeds m^{-2}). Values are for individual plots across N fertiliser treatments at Bull Park and College Field 2014. Predictions of grain N concentration were made using model 4 (c) developed from two sites in 2014, using canopy N content, biomass and soil mineral N content at ear emergence as explanatory variables. RMSE = 0.114. 1:1 line is shown for reference. Slope of the line: y = 0.9625x + 0.085; R^2 = 0.80.

4.3.7.2 Accuracy across sites, year and varieties using models developed from 2013 and 2014 data

The data used to test the predictions from the commercial crops is summarised in Table 4.11. These data provide a wide spread of outcomes with which to test the models with grain N concentration ranging from well below that acceptable for malting (1.11% N) to above (1.91% N). There was also a wide range in yield from 6.1 to 9.0 t/ha @100% DM. Description of site location and crop husbandry details are provided in Appendix 3 for both experimental and commercial crops.

Table 4.11. Ear emergence measurements and harvest grain N concentration from the commercial crops in 2015

Site	Variety	GS 59				Harvest
		N Content (kg ha^{-1})	Biomass (kg ha^{-1})	Potential grain sites (m^{-2})	Soil N (kg ha^{-1})	Grain N concentration (%)
1	**Taberna**	169.64	10121	14367.0	32.75	1.43
2	**Taberna**	173.62	11045	13654.9	35.91	1.75
3	**Taberna**	170.52	10120	9268.4	33.00	1.58
4	**Taberna**	159.95	9229	8925.5	32.11	1.53
5	**Taberna**	172.40	11088	11426.1	32.28	1.65
6	**Taberna**	152.43	11267	13254.1	34.52	1.56
7	**Taberna**	154.08	10097	10185.0	37.54	1.56
8	**Taberna**	133.13	8816	10182.3	32.49	1.61
9	**Taberna**	164.83	11380	11846.0	44.18	1.66
10	**Taberna**	96.81	5651	7040.8	40.59	1.49
11	**Taberna**	71.82	5208	5433.8	41.91	1.44
12	**Taberna**	118.68	7190	8724.9	17.81	1.47
13	**Taberna**	150.94	7171	9640.3	29.47	1.79
14	**Taberna**	158.44	10160	8611.2	43.27	1.59
15	**Propino**	132.56	9154	9753.4	53.64	1.40
16	**Propino**	147.02	9858	10930.6	27.61	1.63
17	**Propino**	99.09	7752	7301.2	28.15	1.20
18	**Propino**	93.68	6380	7729.1	30.12	1.37
19	**Propino**	158.69	10598	11029.5	36.09	1.50
20	**Propino**	110.55	9285	6057.8	37.03	1.15
21	**Propino**	189.04	11512	12297.4	20.22	1.91
22	**Propino**	113.85	8290	12931.8	41.55	1.38
23	**Propino**	116.76	8869	12573.0	29.33	1.28
24	**Propino**	146.06	10967	10965.2	33.12	1.28
25	**Sannette**	93.36	7359	7159.7	35.43	1.11
26	**Sannette**	77.26	7315	7155.5	33.66	1.27
27	**Sannette**	120.01	8933	10240.6	47.80	1.23
28	**Sannette**	129.58	8340	9508.0	36.55	1.57
29	**Sannette**	106.24	8459	7227.3	47.75	1.42
30	**Sannette**	125.07	8174	9129.5	36.63	1.36
31	**Sannette**	96.63	7826	6931.4	32.60	1.11
32	**Sannette**	145.79	12036	11033.6	48.61	1.15
33	**Sannette**	134.88	9901	8562.5	18.71	1.44
34	**Sannette**	138.33	10395	11673.3	39.53	1.31

Models 1-3, developed using data from three sites across two years, 2013 and 2014, were used to predict grain N concentrations from crop measurements at ear emergence in 2015 and their accuracy tested against measured grain N concentrations. Predictions made using model 1b based on measurements of N content and biomass at ear emergence, were poor (Figure 4.3). Although the regression between actual and predicted grain N concentration accounted for a reasonable amount of the variance (R^2=0.60), the slope of the line was appreciably less than 1.0 indicating a large over prediction of N concentration at the upper end of the range. As a result the RMSE is quite large indicating a poor goodness of fit. The inclusion of GS 59 soil mineral N content in the model (Model 1c; Figure 4.4) in conjunction with the other variables increased the amount of variation accounted for (R^2=0.65), but additionally, also improved the slope of the relationship (closer to 1.0) and reduced the RMSE compared to model 1b (Figure 4.3). However, the model still led to an over prediction of grain N concentration, but more consistently over the whole range than model 1b as shown by the displacement of the points from the 1:1 line.

Model 2 substitutes potential grain site number for GS 59 biomass as an explanatory variable. With ear emergence N content and ear emergence potential grain site number as the explanatory variables (Model 2b) the precision of predictions of grain N concentration was reasonable, but the accuracy poor (Figure 4.5). Thus predicted N concentration accounted for a large amount of the variation in measured concentration (R^2=0.74), but there was again a marked over prediction across the range leading to a large RMSE. Including soil mineral N content at GS 59 in the prediction model (Model 2c) improved the goodness of fit (reduced the RMSE), but the predictions were weak (slope = 0.62, R^2=0.18) (Figure 4.6).

Model 3(b) used a combination of N content, biomass and potential grain sites at GS 59 as explanatory variables. Predictions using this model were again poor (slope = 0.53, R^2=0.54, RMSE = 0.37; Figure 4.7). Including ear emergence soil mineral N content in the prediction model (Model 3c) improved the accuracy of predictions substantially (slope = 0.94, R^2=0.62, RMSE = 0.24; Figure 4.8).

4.3.7.3 Accuracy across sites, year and varieties using models developed from 2014 data only

Model 4b was developed by using N content and biomass measurements at ear emergence from two sites in 2014. Predictions of grain N concentration using this model accounted for 80% of the variation in actual N concentration measured across sites, varieties and fertiliser N supplies in 2015 (Figure 4.9). The slope of the relationship between measured and predicted N concentration was close to 1.0, but there was a small over prediction across the range of N concentrations resulting in a small RMSE (0.22).

Inclusion of soil mineral N content as an additional variable (Model 4c) altered the accuracy of the predictions little (RMSE = 0.21) (Figure 4.10). However, the slope of the relationship between actual and predicted grain N concentration was increased to 1.24 indicating a tendency to over predict at low N concentrations and under predict at high concentrations.

The number of potential grain sites was utilised in place of GS 59 biomass for the development of Model 5. Predictions of grain N concentration using model 5b based on N content and potential grain sites at ear emergence as measured variables were reasonably accurate and precise (Figure 4.11; RMSE = 0.24, slope 1.04, R^2=0.71). Including soil mineral N content as an additional variable in the model had minimal effect on the accuracy of predictions (Figure 4.12). Similarly, model 6b based on measurements of ear emergence N content, biomass and potential grain sites, gave accurate and precise predictions of grain N concentration (Figure 4.13; RMSE = 0.23, slope = 1.07, R^2=0.79). There was only a small improvement in RMSE and R^2 (Figure 4.14) when soil mineral N content were included in the prediction (Model 6c), but the slope of the relationship between predicted and N actual grain N concentration was increased to 1.20.

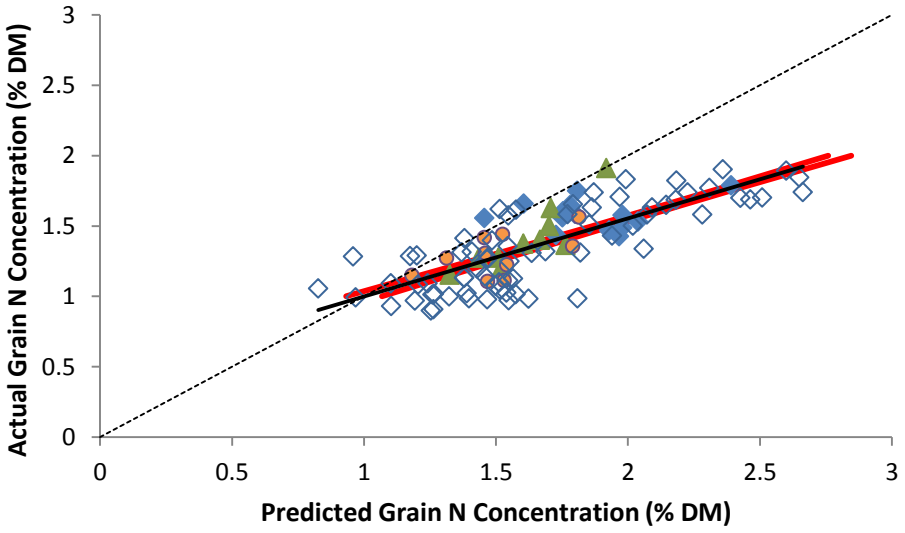

Figure 4.3. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 1 (b), which was developed using data from three sites across two years with N content and biomass at ear emergence as explanatory variables. RMSE= 0.422; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 0.555x +0.445; R^2 = 0.60. ——— = Confidence Interval

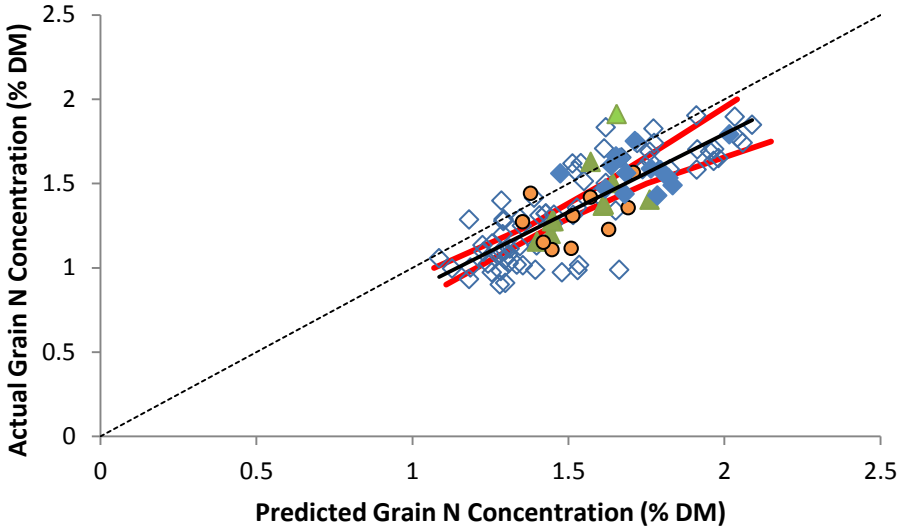

Figure 4.4. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 1 (c), which was developed using data from three sites across two years with N content, biomass and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.278; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino. (●) Sanette. commercial crops. Slope of the line: y = 0.927x - 0.0593; R^2 = 0.65. ——— = Confidence Interval

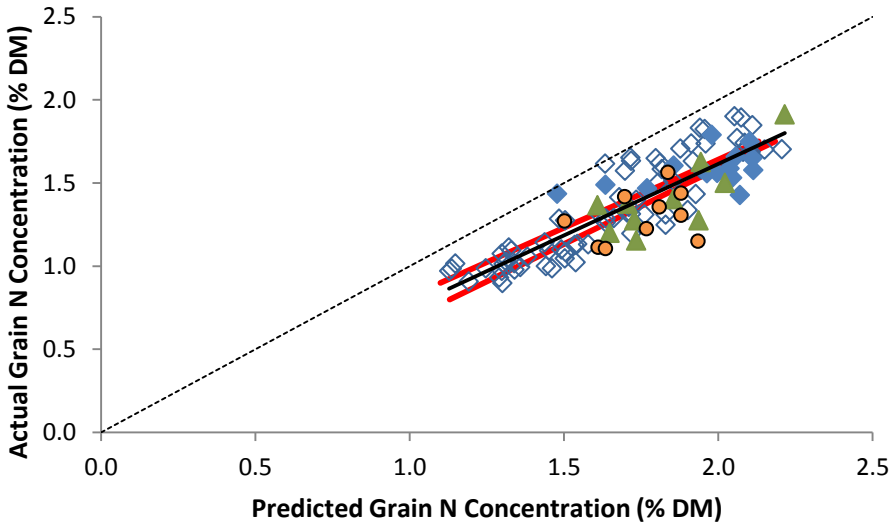

Figure 4.5. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 2 (b), which was developed using data from three sites across two years with N content and potential grain sites at ear emergence as explanatory variables. RMSE= 0.420; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 0.860x – 0.106; R^2 = 0.74. ——— = Confidence Interval

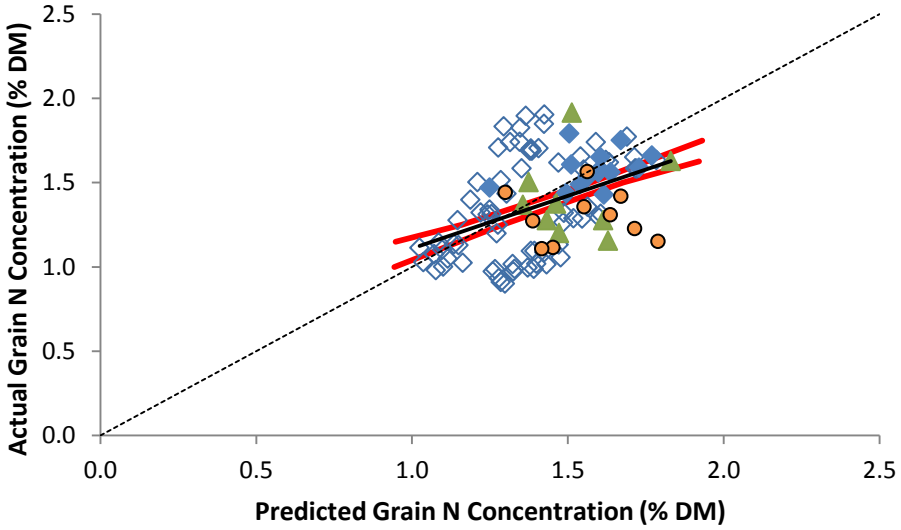

Figure 4.6. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 2 (c), which was developed using data from three sites across two years with N content, potential grain sites and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.291; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 0.624x – 0.485; R^2 = 0.185. ——— = Confidence Interval

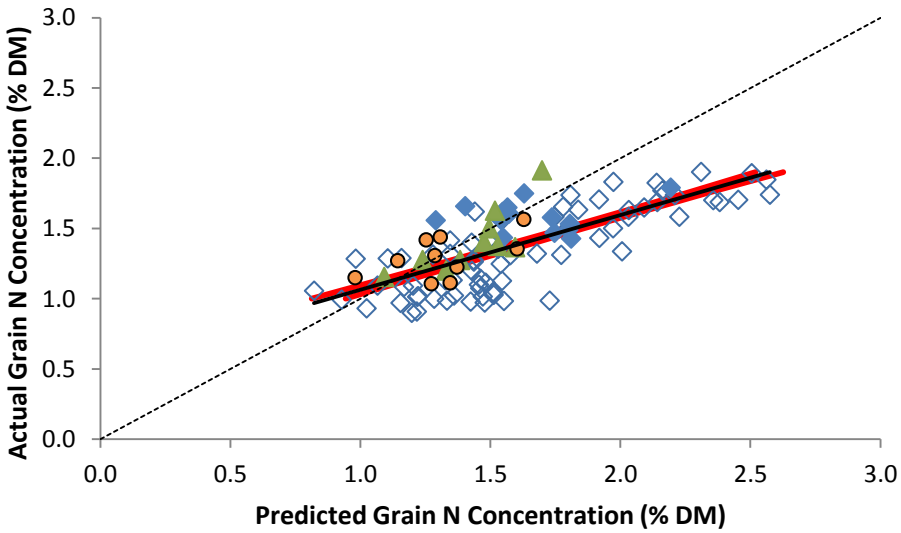

Figure 4.7. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 3 (b), which was developed using data from three sites across two years with N content, biomass and potential grain sites at ear emergence as explanatory variables. RMSE= 0.371; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 0.531x – 0.534; R^2 = 0.54. ——— = Confidence Interval

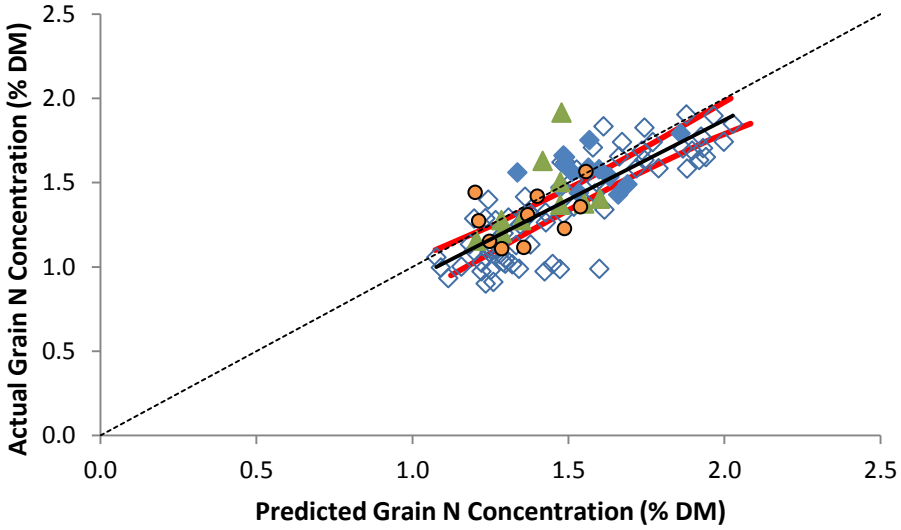

Figure 4.8. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 3 (c), which was developed using data from three sites across two years with N content, biomass, potential grain sites and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.243; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 0.944x – 0.016; R^2 = 0.62. ——— = Confidence Interval

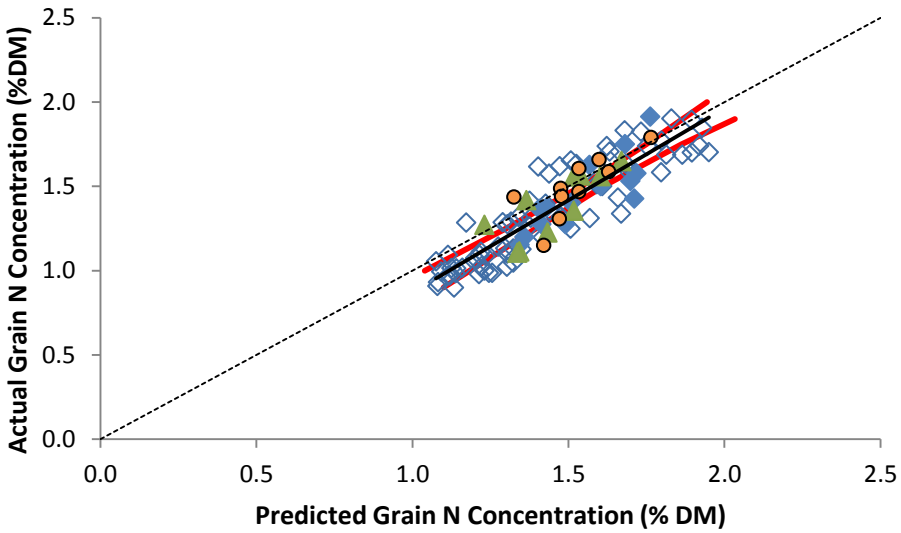

Figure 4.9. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 4 (b), which was developed using data from two sites in 2014 with N content and biomass at ear emergence as explanatory variables. RMSE= 0.220; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.092x – 0.528; R^2 = 0.80. ——— = Confidence Interval

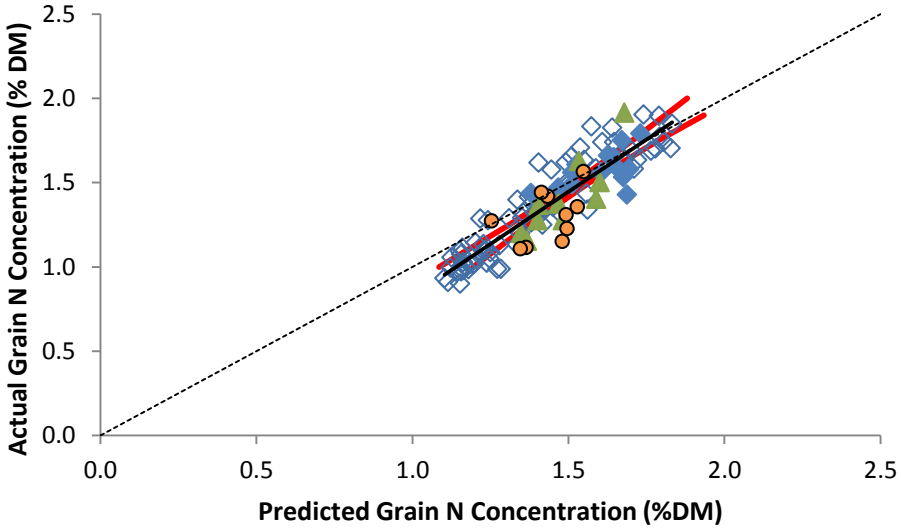

Figure 4.10. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 4 (c), which was developed using data from two sites in 2014 with N content, biomass and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.208; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.238x – 0.412; R^2 = 0.82. ——— = Confidence Interval

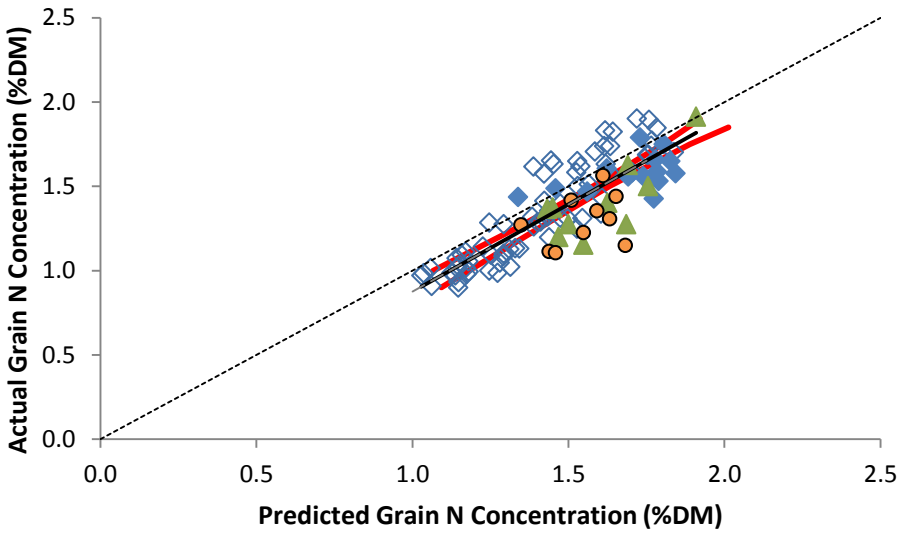

Figure 4.11. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 5 (b), which was developed using data from two sites in 2014 with N content and potential grain sites at ear emergence as explanatory variables. RMSE= 0.243; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.037x – 0.163; R^2 = 0.71. ——— = Confidence Interval

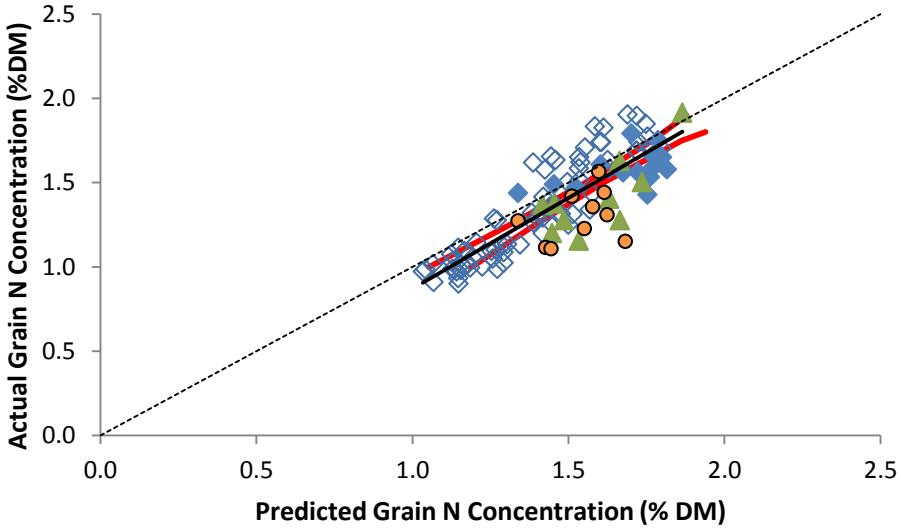

Figure 4.12. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 5 (c), which was developed using data from two sites in 2014 with N content, potential grain sites and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.236; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.079x – 0.210; R^2 = 0.71. ——— = Confidence Interval

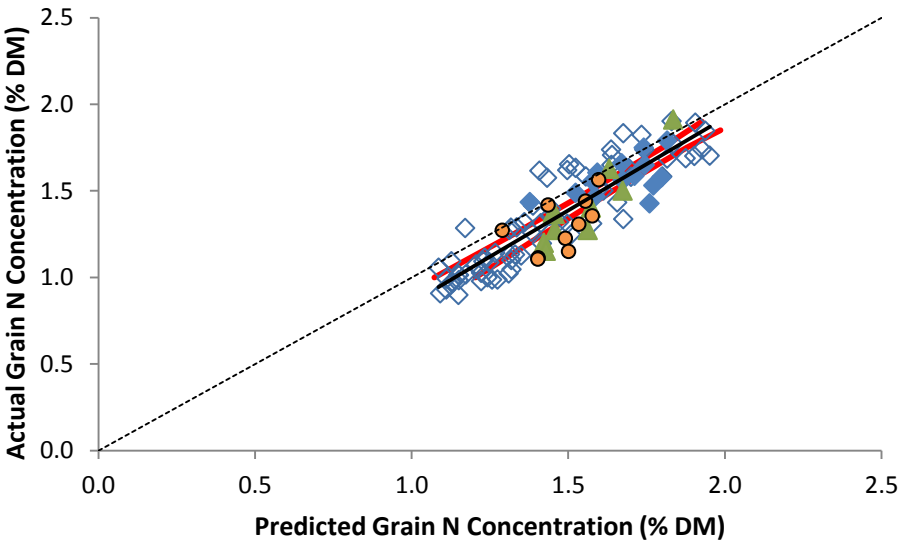

Figure 4.13. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 6 (b), which was developed using data from two sites in 2014 with N content, biomass and potential grain sites at ear emergence as explanatory variables. RMSE= 0.233; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.068x – 0.216; R^2 = 0.79. ——— = Confidence Interval

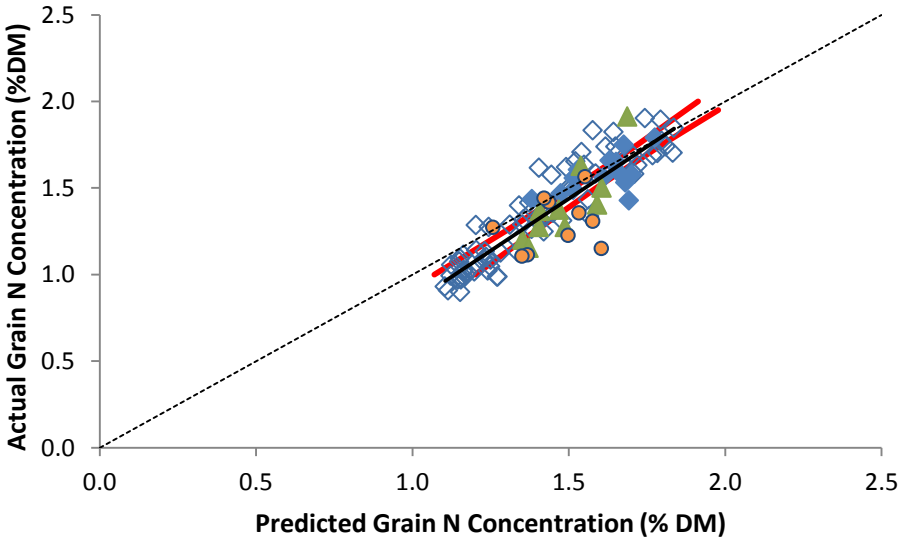

Figure 4.14. Actual versus predicted values of grain N concentration produced from the validation data set (2015) using Model 6 (c), which was developed using data from two sites in 2014 with N content, biomass, potential grain sites and soil mineral N content at ear emergence as explanatory variables. RMSE= 0.211; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◊) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.204x – 0.367; R^2 = 0.80. ——— = Confidence Interval

4.3.7.4 Confidence and Prediction Limits

The upper and lower 95% confidence and prediction limits are shown in Table 4.12 for each of the models. Model 4b is examined in more detail as it exhibits the greatest level of variation accounted for, a small RMSE indicating a strong goodness of fit and the slope is close to 1, which would suggest it may perform as the best prediction model. The confidence interval limits show that at the approximate mid-range grain N concentration found in barley crops of 1.5%, it can be predicted to within ± 0.035% of the actual grain N concentration, although an average bias by 0.1% overestimation. If a crop at ear emergence is predicted to have, on average, grain N concentration of 1.5% ± 0.035%, producing a range between 1.465 and 1.535% grain N, the actual grain N concentration observed at harvest would be 1.41% demonstrating the consistent average bias of 0.1% overestimation. The confidence limits are slightly larger at both ends of the range of predictions; however the margin of error still remains under ± 0.1%. The prediction limit, based on one single prediction, is much larger than that of the confidence limit. In a similar scenario of a predicted grain N concentration of 1.5% with a margin of error of ± 0.35% grain N concentration; the predicted grain N concentration could be between 1.15 and 1.85%. However, the actual grain N concentration achieved was 1.41%, representing the consistent over prediction of 0.1%.

Table 4.12. 95% Confidence and Prediction Intervals of models at a range of predicted grain N concentrations

		Predicted Grain N Concentration (% DM)				
Model	**Interval**	**1**	**1.25**	**1.5**	**1.75**	**2**
1b	Confidence	0.068	0.050	0.037	0.034	0.044
	Prediction	0.348	0.345	0.344	0.343	0.344
1c	Confidence	0.078	0.050	0.032	0.041	0.067
	Prediction	0.329	0.323	0.321	0.322	0.327
2b	Confidence	0.076	0.054	0.035	0.027	0.038
	Prediction	0.286	0.281	0.278	0.278	0.279
2c	Confidence	0.118	0.065	0.051	0.095	0.153
	Prediction	0.503	0.493	0.492	0.498	0.513
3b	Confidence	0.065	0.048	0.037	0.039	0.053
	Prediction	0.372	0.358	0.368	0.368	0.370
3c	Confidence	0.147	0.089	0.064	0.100	0.160
	Prediction	0.342	0.336	0.335	0.337	0.343
4b	Confidence	0.080	0.048	0.035	0.057	0.090
	Prediction	0.365	0.358	0.358	0.361	0.368
4c	Confidence	0.055	0.031	0.023	0.041	0.067
	Prediction	0.238	0.233	0.232	0.235	0.240
5b	Confidence	0.068	0.041	0.029	0.045	0.073
	Prediction	0.300	0.296	0.294	0.296	0.302
5c	Confidence	0.069	0.041	0.029	0.047	0.077
	Prediction	0.301	0.295	0.294	0.296	0.302
6b	Confidence	0.057	0.033	0.024	0.042	0.068
	Prediction	0.252	0.248	0.247	0.248	0.252
6c	Confidence	0.057	0.035	0.024	0.036	0.059
	Prediction	0.247	0.243	0.242	0.244	0.250

4.4 **Discussion**

Grain N concentration is the quotient of two components; grain yield and grain N content. To accurately predict grain N concentration, it is necessary for models to include explanatory variables which can account for the variation that occurs in each of these components across sites, seasons and crop management practice. A good forecasting system must be a financially feasible, time efficient and robust method, that delivers an acceptable accuracy of prediction. Accuracy is important as subsequent management decisions may be influenced by the prediction and ultimately, may affect producer profitability. Predictions must be tested using independent measurements made under the same crop management, soil and climatic conditions the forecasting system is intended to be used in.

In general, the most cost effective forecasting system will be one that provides an acceptable level of accuracy across environments and management regimes with the smallest number of explanatory variables**.** All models tested included a minimum of two explanatory variables; one that related strongly to grain N content, the other to grain yield. For the latter biomass or the number of potential grain sites at ear emergence were used. In some models both were included to investigate whether combining these variables provided a more robust prediction.

Models developed from data from three site-seasons (2013 and 2014), generally gave poor predictions of grain N concentration. A possible reason for this can be related to seasonal weather conditions. The 2013 season was cooler than average at the start of the year and coincided with rainfall figures much below the long term average. This data is supported by the evidence of soil moisture deficits as shown in Table 3.3, as high plant available water depletion was over 60%. The combination of these factors occurring at early vegetative growth stages had a major impact on biomass production, as biomass production has been shown to be inhibited by water stress. Water stress has been documented to reduce leaf area expansion (Farooq *et al.*, 2009) in turn reducing solar radiation use efficiency with the resultant outcome being decreased growth rate and ultimately reduced biomass accumulation (Jamieson *et al*., 1995). By contrast, the 2014 season was much closer to the long term average in

terms of temperature, with rainfall averaged out across the year. Weather conditions in 2015 were typical of barley production environments in Ireland and comparable to those of 2014. Thus it is not surprising that models developed using data from two sites in 2014 in general provided better predictions than those that included measurements made in an atypical year.

The growth and development of both wheat and barley pre and post anthesis impact on grain yield and grain N content and ultimately, the nitrogen concentration of the grain as cereal crops depend on a number of sources for grain filling, including current assimilation and mobilisation of reserve carbohydrates and transport of N from vegetative mass and concurrent N uptake and the subsequent transport to the grains (Schnyder 1993; Simpson, 1983; Kichey *et al*., 2007). Stress factors both pre and post anthesis, such as high temperature, moisture stress, limited solar radiation and disease can have significant effects on the deposition of nitrogen and carbohydrate to the grain. Grain number per unit area and hence grain yield has been shown to be linearly related to a crops' intercepted solar radiation levels pre-anthesis (Fischer, 1985). Savin and Slafer (1991) found that grain yield was reduced due to decreases in both grain weight and grain number m^{-2} in response to shading from anthesis to maturity simulating reduced solar radiation. The optimum temperature range for grain fill is 15-18°C and temperatures greater than 30°C during the post-anthesis period may inhibit the duration of grain filling, while this reduction is not compensated for by increased rates of grain fill, resulting in reduction of grain weight (Dias and Lidon, 2009; Wardlaw *et al*., 1980; Bhullar and Jenner 1986; Stone *et al*., 1995). The reduction in starch deposition caused by such high temperatures during grain fill affects grain N concentration by altering the ratio of nitrogen to carbohydrate per grain (Gooding *et al*., 2003; Stone and Nicolas, 1998). Post anthesis drought reduces the duration of grain filling and ultimately grain yield through reduced grain weight (Nicolas *et al*., 1985; Mogensen, 1992). Drought conditions have also been found to reduce grain weight to a greater extent than high temperatures (Savin and Nicolas, 1996).

Models developed using data from N response experiments conducted in 2014 at the standard commercial seed rate gave good predictions of grain N concentration

observed in a lower seed rate in the same year (Figure 4.1 and 4.2 - RMSE = 0.251; R^2 = 0.75 and RMSE = 0.114; R^2 = 0.80 respectively). This is in spite of a reduction in grain yield and an increase in grain N concentration after lowering the seed rate. Although seed rate was reduced to half of standard agronomic seed rate, the reduction in grain yield was small at only 0.3 t ha^{-1} (when averaged across sites and N rates). Several researchers have conducted work examining the effect of seed rate on grain yield with results suggesting little or no effect on grain yield, including Conry, (1998); Gonzalez Ponce *et al. (*1993); Spaner *et al. (*2001). The response to seed rate is consistent with that of Gooding *et al. (*2002), whereby the crops compensated for low plant population densities through increased tiller production and survival in association with increased grain number per ear. Grain N concentration was also higher in the low seed rate treatment, agreeing with the findings of O'Donovan *et al*., 2011. This increase in grain N concentration was explained by the small reduction in grain yield by lowering seed rate and the absence of an effect of seed rate on grain N content. The results suggest that grain N concentration might be predicted with reasonable accuracy across crops grown at different seed rates or where there has been poor establishment within the range found in normal commercial practice.

The models developed from the three site-season combinations produced poor predictions of validation data, due to the abnormal growing conditions in 2013, given that these conditions are rare in Ireland, models developed from two sites from 2014 with more normal conditions will be discussed and evaluated further. Although models developed used measurements of crop variables at ear emergence, many researchers use anthesis as a benchmark for analysis. It is important to acknowledge the sequence of growth stages and proximity to one another (ear emergence and anthesis) as in spring barley crops, anthesis may precede complete ear emergence (Spink *et al*., 2015). Anthesis accumulated N can represent between 60 and 90% of total N found in the crop at maturity in winter and spring cereals, including wheat and barley (Austin *et al*., 1977; Heitholt *et al*., 1990; de Ruiter and Brooking, 1994; Bulman and Smith, 1994; Palta and Fillery, 1995 and Kichey *et al*., 2007). Furthermore, van Sandford and MacKown (1987) and Bulman and Smith (1994) highlighted the importance of post anthesis N uptake in total crop N uptake at

harvest. In the data presented here, however, soil mineral nitrogen was depleted to minimal levels by ear emergence (Figure 3.7) by the crops large nitrogen uptake requirements, which were illustrated by high biomass accumulation and large canopy size (Figures 3.5 and 3.6). Soil mineral N contents were higher at the highest fertiliser N supply rates where applications were above optimum N levels, but these fertiliser input levels would be significantly above those permitted by legislation and unrepresentative of crops grown for malting (See Appendix 4 for details). Therefore, whilst soil mineral N contents were available for post anthesis N uptake, its contribution to final crop N content was relatively small.

Total biomass production at maturity and the partitioning of assimilate between grain and non-grain material determines final grain yield (Van den Boogaard *et al*., 1996). However, harvest index was relatively constant and accounted for a very small proportion of variation in yield (Figure 3.18), similar to findings of White and Wilson (2006), illustrating that variation in yield was largely accounted for by variation in total biomass in agreement with Cossani *et al. (*2009). Total biomass at anthesis and maturity were shown to be correlated by Austin *et al. (*1977) and Przulj and Momcilovic (2003), also found that in a range of environmental conditions and nitrogen fertiliser applications, approximately 50% of total dry matter at maturity was present at anthesis. This has been proven in both wheat (Turner, 1997) and barley (Ramos *et al*., 1985).

Cereal crop grain yield is the product of two components – grain number and grain weight. Grain number in barley is highly correlated with yield (Gallagher *et al*., 1975; Baethgen *et al.*, 1995; Abeledo *et al.*, 2003; del Moral *et al.*, 2003; Blake *et al.*, 2006; Bingham *et al.*, 2007a). During the pre-anthesis period of vegetative growth, potential grain number per unit area is determined (Fisher, 1985; Gallagher *et al.* 1975; Slafer *et al.* 2009). Sinclair and Jamieson (2006) provide a detailed description of the process that determines potential grain sites and grain number and the factors affecting it. Ultimately, they describe the critical stage for a potential grain site to set is to have pollen and ovules fully formed by spike emergence (Langer and Hanif, 1973 and Sibony and Pinthus, 1988). However, the number of potential sites for grain production greatly exceeds the number of grains which

actually develop. This can be affected by many factors including N availability in the spike (Sinclair and Jamieson, 2006). Although potential grain sites are significantly related to grain number at harvest, it accounts for a small level of variation (0.31); which could be explained by factors affecting grain set of potential sites. This equates with the findings here that prediction equations using total biomass at ear emergence as a predictor of final yield, generally gave better predictions than those using potential grain number. Using both total biomass at ear emergence and potential grain number combined into one prediction equation did not improve the prediction over those using total biomass alone.

Including soil mineral N content at ear emergence in the prediction equations gave very minimal improvements, if any, in the percentage variance accounted and tended to give predictions with a slope greater than the 1:1 line.

Overall equation 4b which used total crop N content and biomass at ear emergence and was derived from the 2 sites in 2014 gave the best prediction of grain N concentration from the validation data collected in 2015 and for the low seed rate plots in 2014. It also has the distinct advantage over other models in that the data required to make a prediction are relatively easy to acquire, and has the potential for the data to be collected remotely using sensor based methods.

The prediction interval essentially spans the range of possible N concentrations from the minimum to maximum thresholds, and therefore offers no use as a management tool. Because the prediction of grain N concentration using a single sample at ear emergence had wide prediction limits, grain N concentration would be best estimated from an average of a number of individual predictions of grain N concentration. Reasonable averages of grain N concentration should be achieved from a field, farm or region, through approximately 5 samples, generally increasing the number of individual measurements above this has a minimal impact on the accuracy of the mean. However, this prediction model performs under the caveat that the weather conditions are similar to long term averages, and no post measurement stress such as drought or late season disease occurs to affect grain fill. It does however provide the possibility of the model being used both tactically and strategically by producers and maltsters respectively.

Whilst the best predictive model was developed from only one growing season's primary data and on one variety, the fact that it performed well over a range of varieties and sites the following season gives some confidence that it should be reasonably robust. However, further development of the model with a larger pool of data across a greater number of seasons, sites and varieties would encourage even greater confidence in the predictions produced.

Foliar N application can increase grain N concentration more than soil applied N as uptake is less dependent on soil moisture conditions (Dampney and Salmon, 1990) in late season when grain filling occurs. Gooding *et al. (*2007) conducted research into the recovery of nitrogen from foliar applied N (urea) and soil applied granular (ammonium nitrate) at anthesis to wheat crops that had previously received 200 kg N ha^{-1}. Recovery of N in the grain where anthesis N was applied differed depending on N source. Foliar N recovery was on average almost 22% while granular N was lower at only 15% recovery. As neither method of application significantly affected grain yield and increased grain N content, grain N concentration was significantly increased. The results of this study show significantly higher rates of recovery of anthesis applied N than in previous work e.g. Gooding *et al. (*2007), which may be as a result of the generally higher levels of late season soil water supply in Ireland's moist climate. Anthesis N application resulted in grain N concentration increases of 0.2% following the application of 30kg N ha^{-1} and 0.3% following the application of 40kg N ha^{-1}, although as these were applied in different seasons the increase in grain N concentration cannot be directly related to the rate of N applied.

The results indicate that in crops or seasons where grain N concentration is predicted to be low it would be worthwhile applying 30-40 kg N ha^{-1} at anthesis where grain destined for the brewing market is predicted to be 1.2-1.5% N or for the distilling market between 1.0 and 1.3%.

5 The potential for using non-destructive measures of N content and biomass at ear emergence to predict grain N concentration

5.1 Introduction

Prediction of grain N concentration of malting barley during the growing season could inform producer management decisions regarding inputs of fertiliser N as well as prioritise harvesting. It would also provide useful information for maltsters regarding the quality of a crop in a particular year.

Results in Chapter 4 indicated that measurements of crop biomass and crop N content when the crop had reached full ear emergence could be used to give good predictions of grain N concentration at harvest. However the measurement of both crop biomass and crop N content is labour- and time-consuming, requiring multiple processes as well as access to specialised laboratory facilities. Therefore a prediction system that relies on these inputs is unlikely to be of any practical use unless less time- and labour-intensive means of acquiring estimates of these parameters can be found.

Non-destructive measures have the potential to provide cost-effective, rapid and non-invasive estimates of measured crop variables (Montes *et al*., 2007). A range of non-destructive techniques exist. Canopy reflectance, which utilises radiation reflected from plant canopies, has the potential to indicate both biomass and the N status of crops (Daughtry *et al*., 2000; Fiella *et al*., 1995; Portz *et al*., 2013; Babar *et al*., 2006). Canopy reflectance measurements have also been related directly to grain N concentration (Pavulur *et al*., 2015). LAI has been shown to have strong correlations with biomass (Petcu *et al*., 2003) and canopy N content (Yin *et al*., 2003).

Canopy reflectance can be determined using sensors that are carried on a range of platforms including satellites, aircraft, unmanned aerial vehicles and ground vehicles including tractors as well as being hand held. The use of satellite and aircraft-mounted systems facilitates the large scale coverage of crops; however, their use can

be limited by cloud cover (Grenzdörfer, 2001). Another key advantage of reflectance measurements is that results are available instantaneously or soon after measurement.

The chlorophyll meter (such as the Minolta SPAD-502) provides a simple, rapid and non-destructive method of estimating leaf chlorophyll N content (Watanabe *et al*., 1980), which has been shown to be strongly correlated to N (Evans, 1983). However, SPAD values are also influenced by leaf optics, which may be affected by environmental and biological factors (Manetas *et al*., 1998; Markwell *et al*., 1995). Chlorophyll meters calculate readings based on the amount of absorbed red light (650nm) by chlorophyll and the absence of absorbed infrared light (940 nm) by chlorophyll (Markwell *et al*., 1995). Despite the advantages outlined, the scanning of individual leaves is time consuming and values produced from the chlorophyll meter are dimensionless. Without calibration, chlorophyll meters have restricted use, as they only display relative values of chlorophyll content (Peterson *et al*., 1993; Olfs *et al*., 2005). As it has been shown that nitrogen limitation decreases chlorophyll content (Peñuelas *et al*., 1994), when severe nitrogen deficiency is present and thus reduced chlorophyll content, plants reflect a greater amount of the red spectral region. Additionally, changes in soil cover and plant density caused by N stress are confounded with changes in vegetation colour (chlorosis) (Steven *et al*., 1990). Rapid, non-destructive predictions of grain N concentration early in season would afford producers a management tool for grain N management. Nitrogen management may result in late season fertiliser N, as Rawluk *et al. (*2000) found that grain N concentration increases consistently across a wide range of conditions with anthesis N applications.

Grain N concentration prediction models were developed in Chapter 3 and a validation test was conducted in Chapter 4 to determine the most accurate prediction across site, years and varieties. It also investigated the extent of change in grain N concentration that can be achieved using late season fertiliser N applications of crops predicted to have low grain N concentration. These prediction models rely on accurate measures of N content and biomass at ear emergence.

Remote sensing, which utilises radiation reflected from plant canopies, has the potential of evaluating the N status of many plants within the field of view of the sensor (Daughtry *et al*., 2000; Fiella et al., 1995). Its ability to estimate crop N status using high spatial resolution across a wide plant community compared to single plant analysis is recognized as a superior advantage (Bausch *et al*., 1997). Remote sensing uses near infrared (NIR) and visible (VIS) light to calculate crop canopy reflectance. However, reflectance wavelengths can be very sensitive to biomass, variable irradiance, background effects and the arrangement of sensor (Curran, 1983; Munden *et al*., 1994). The use of satellite and aircraft-mounted systems facilitates the large scale coverage of crops; however, their use is limited as they are reliant upon weather conditions of cloud and interference (Grenzdörfer, 2001).

Leaf area index (LAI) is the total one sided area of leaf per unit of ground surface area which is expressed as a dimensionless variable (Watson, 1947). Direct measures of leaf area are costly, time-consuming and destructive (Marshall, 1968). The most direct, destructive and labour intensive method involves the physical removal and measurement of leaves (Bréda, 2003). Sestak *et al. (*1971) provided a detailed account of methodology of various practices of leaf area estimation. Optical methods of indirect non-contact and non-destructive estimation of LAI are based on the measurement of light transmission through the canopy, such as the GreenSeeker (Trimble Inc., Sunnyvale, CA, USA), CropSpec (Topcon Positioning Systems, Inc., Livermore, CA, USA) or CropCircle (Holland Scientific Inc., Lincoln, NE, USA). This method measures incident photosynthetic active radiation (PAR) and transmitted PAR above and below the canopy (Bréda, 2003).

The objectives of this study were to examine 1) the relationship between non-destructive measurements and both crop biomass and canopy N content after the completion of heading, 2) the ability of estimates of crop biomass and canopy N content derived from non-destructive measurements to predict grain N concentration, 3) the direct relationship between non-destructive measurements and grain nitrogen concentration, 4) the effect of canopy N distribution on canopy reflectance and leaf chlorophyll content.

5.2 Materials and Methods

5.2.1 Model development

Three experiments, one in 2013 and two in 2014, each with a range of fertiliser N rates were carried out as detailed in Chapter 3.2.2. At crop maturity grain yield, grain N concentration, total crop N uptake and yield components were determined. At the completion of ear emergence (GS 59) biomass, N accumulation, GAI, NNI, tissue N concentration and soil mineral N reserves were determined. Methodology was as detailed in Chapter 3.2.4. In addition, measurements of canopy reflectance and estimates of LAI, calculated from light interception measurements, were made at the ear emergence sampling time, on plots designated for combine harvest i.e. plots that had not been destructively sampled.

Canopy reflectance was measured using a hand held Crop Circle ACS 470 instrument (Holland Scientific Inc. Lincoln, NE, USA) which is an active sensor emitting white light (~400 to ~800 nm) and measuring reflectance in three user determined wavelengths. For this study reflectance was determined at 760, 730 and 670 nm. The instrument was set to make five measurements per second. At each measurement date the instrument was moved along the centre of a plot with a nadir view of the crop at a height of ~0.7m above the top of the crop canopy, avoiding 1.5 m at each end of the plot. This gave rise to 25-35 measurements at each wavelength per plot.

The normalised difference vegetation index, calculated using the red edge band, NDRE was calculated as

Normalized Difference Red Edge (Barnes *et al*., 2000)

$$NDRE = \frac{(R760 - R730)}{(R760 + R730)}$$

where R760, R730 refer to reflectance at 760nm and 730nm respectively.

Light interception was determined at each sampling date by simultaneously measuring radiation both above and below the canopy using a SunScan Canopy Analysis System (Delta-T Devices, Cambridge, UK) in combine harvest designated plots. A total of 6 measurements were taken per plot. Measurements were taken between 11 am and 2 pm. The SunScan probe was inserted at an angle of 45° to the direction of the crop rows. LAI was calculated from interception data as measurements were being made using the SunScan algorithm (Webb *et al.*, 2014). The Ellipsoidal Leaf Angle Distribution Parameter (ELADP) was set at 1 (Annon, 2015).

5.2.2 **Model validation**

The developed models were validated using data collected from two nitrogen response experiments, carried out in 2015 and data collected from a range of commercial crops in 2015.

Details of the two nitrogen response experiments are given in Chapter 4.2.3. Briefly, the experiments included a range of fertiliser N rates (0, 30, 60, 90, 120, 150, 180, 210, 240 kg N ha^{-1}) applied to the same variety as in the experiments used to provide data for model development.

Briefly, the commercial crops comprised 14, 10, and 10 crops of the varieties Taberna, Propino and Sanette respectively, grown on sites with a range of soil types, a range of previous cropping histories and receiving between 135 and 150 kg N ha^{-1} of fertiliser N.

Both the commercial crops and the nitrogen response experiments were destructively sampled at ear emergence (biomass, crop N uptake) and at harvest (grain N concentration) using methods detailed in Chapter 4.2.4. No combine yields were taken from commercial crops or response experiments. Reflectance measurements and indirect estimates of LAI were determined as described in 5.2.1.

Additional measurements were made at the pre-anthesis sampling date on one of the nitrogen experiments to examine the effect of fertiliser N rate on the distribution of

nitrogen between different leaf layers and consequent effects on canopy and leaf spectral properties. Ten stems were randomly selected within each plot. For each of the top four leaves on each stem measurements were made with a Minolta chlorophyll meter (Konica Minolta, Inc., Osaka, Japan). The Minolta chlorophyll meter produces dimensionless units (SPAD units) that are relative to chlorophyll concentration (Peterson *et al.,* 1993; Olfs *et al*., 2005). The measurements were made on the central part of the leaf blade, while avoiding the leaf midrib, approximately mid-way between the base and tip of the leaf blade.

These randomly selected stems were then transported to the laboratory. After removing the roots the stems were initially separated into ear plus canopy layers. The uppermost layer, layer 1, comprised the flag leaf lamina and stem above the base of the flag leaf lamina. Subsequent layers comprised the next lower leaf lamina, remaining stem above the base of that leaf lamina as well as the leaf sheath enfolding that stem. Plant parts were then milled and N concentration determined following similar procedures to those outlined in Chapter 3.2.4. Subsequently the N content of each plant part was calculated as the product of its N concentration and biomass

5.2.3 **Statistical Analysis**

During model development the relationships between indirect measurements of the crop made at full ear emergence (LAI and NDRE) and grain nitrogen concentration at harvest for data obtained during the 2013 and 2014 seasons were modelled using regression analysis. Both linear and non-linear models were tested for each relationship and where necessary separate models were fitted for different sites. The models giving the highest R^2 was chosen as the most suitable for predicting GNC. Relationships between indirect measurements of the crop made at full ear emergence (LAI and NDRE) and direct measurements of the crop made at full ear emergence (biomass and crop N accumulation) were examined in a similar fashion.

Data obtained from crops grown in 2015 were used to validate the model relating indirect measurements to grain N concentration. Validation was achieved by using indirect measurements (LAI and vegetation index) made at GS 59 to predict grain N concentration and comparing these predicted values with actual values. The

accuracy and precision of the predicted values was determined by plotting actual grain nitrogen concentration versus predicted grain nitrogen concentration. Deviation of the slope and intercept of the linear regression between actual and predicted values from 1 and 0 respectively was determined to assess accuracy. RMSE was used as a measure of precision. The data was analysed using GenStat (14^{th} Edition).

5.3 Results

5.3.1 Relationship between non-destructive measurements and grain N concentration

Figure 5.1 illustrates the relationship between NDRE and grain N concentration (% DM). When data from the three site-seasons were combined a significant ($P<0.001$) linear relationship was found between NDRE and grain N concentration; however the level of variation explained was low ($R^2 = 0.37$). When the model was allowed to have separate site-season coefficients the percentage of variation explained increased to 0.76. There was a significant curvilinear relationship ($P<0.001$) between NDRE and grain N concentration for the Road Field *(a)* site that accounted 98% of variation in NDRE. When data were combined from Bull Park and College Field *(b)*, NDRE accounted for 92% of the variation in grain N concentration ($P<0.001$). There appears to be a saturation effect of NDRE at values of 0.4, which coincided with grain N concentrations of around 1.3 at Bull Park and College Field and around 2 at Road Field.

Figure 5.2 illustrates the relationship between leaf area index and grain N concentration. When data from the three site-seasons were combined a significant ($P<0.001$; $R^2 = 0.69$) overall relationship was found between LAI and grain N concentration. When the model was fitted with separate site-season coefficients the percentage of variation explained increased to 0.84. There was a significant linear relationship ($P<0.001$) between LAI and grain N concentration for the Road Field *(a)* site that accounted for 78% of variation. When data were combined from Bull Park and College Field *(b)*, 88% of the variation was accounted for.

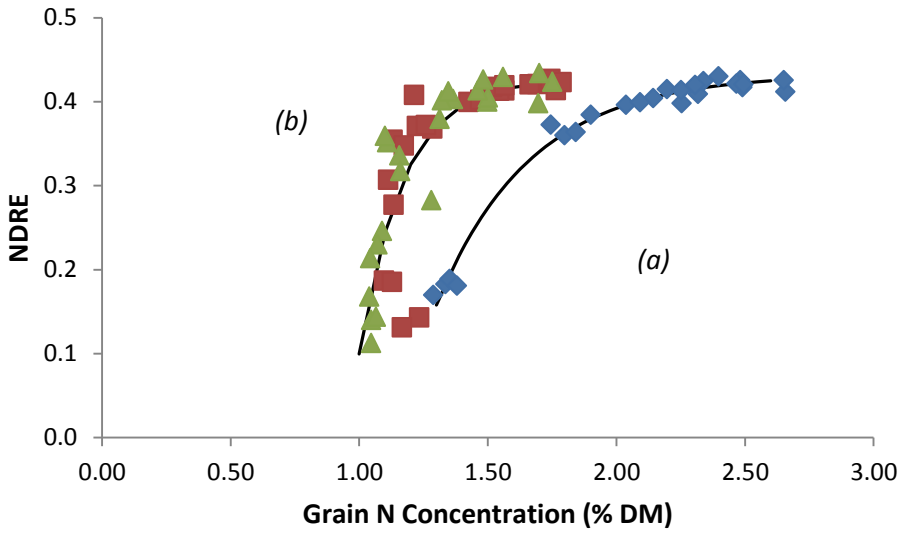

Figure 5.1. Relationship between NDRE and grain N concentration (% DM). (a) Road Field (2013) (◆) ($P<0.001$; $R^2 = 0.98$) and (b) combined Bull Park (■) and College Field (2014) (▲) data ($P<0.001$; $R^2 = 0.97$). Each point represents an individual plot value.

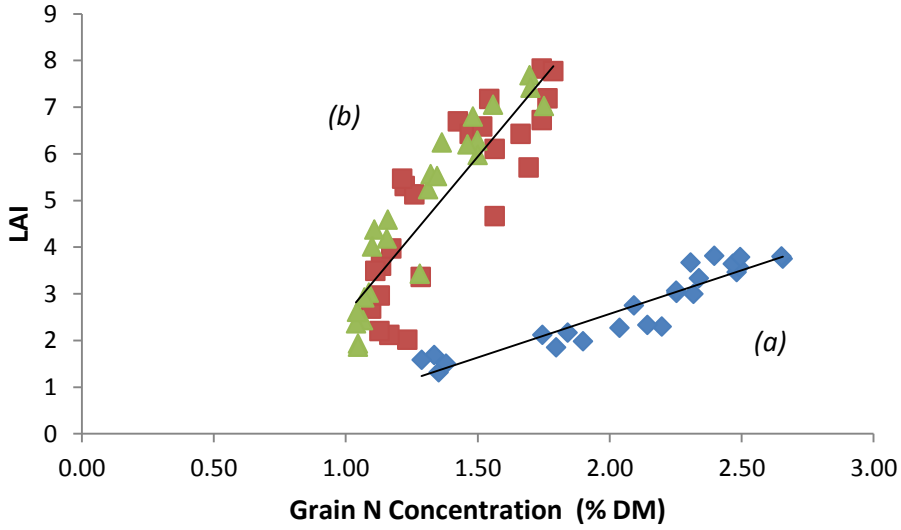

Figure 5.2. Relationship between leaf area index (LAI) and grain N concentration (% DM). (a) Road Field (2013) (◆) ($P<0.001$; $R^2 = 0.78$) and (b) combined Bull Park (■) and College Field (2014) (▲) data ($P<0.001$; $R^2 = 0.88$). Each point represents an individual plot value.

5.3.1.1 Validation of predicted grain N concentrations from non-destructive measures

The relationship between actual and predicted grain N concentration calculated from canopy reflectance (NDRE) measurements at GS59 is shown in Figure 5.3. Predictions of grain N concentration using this model accounted for 48% of the variation in actual N concentration measured across sites, varieties and fertiliser N supplies in 2015. Although the RMSE was reasonable (0.29% N), the relationship was curvilinear ($P<0.001$). As the slope of the line was greater than one, it indicated an under prediction. Figure 5.4 illustrates the predictions of grain N concentration using LAI produced an R^2 of 0.66 and an RMSE of 0.25. However, the slope of the relationship between actual and predicted grain N concentration was 1.4 indicating a tendency to over predict at low N concentrations and under predict at high concentrations.

5.3.1.2 Confidence and Prediction Intervals

As the relationship between actual and predicted grain N concentration calculated using canopy reflectance (NDRE) was curvilinear, it was judged unsuitable as a predictor of grain N concentration. Therefore, confidence and prediction limits were not calculated. When LAI was used to predict grain N concentration, the relationship between actual and predicted values was linear. Table 5.1 shows the upper and lower 95% confidence and prediction limits for grain N concentration predictions derived from LAI. If a crop at ear emergence is predicted to have, on average, a grain N concentration of 1.5% ± 0.055%, the user could be confident that the actual value at harvest would (with 95% probability) lie between 1.45 and 1.56%. The confidence limits are larger at both ends of the range of predictions. However, the prediction limit, based on one single prediction, is much larger than that of the confidence limit. In a similar scenario of a predicted grain N concentration of 1.5% with a margin of error of ± 0.32% grain N concentration; the predicted grain N concentration could be between 1.18 and 1.82%. However, the actual grain N concentration achieved would be 1.7%, indicating an over estimation of 0.2%.

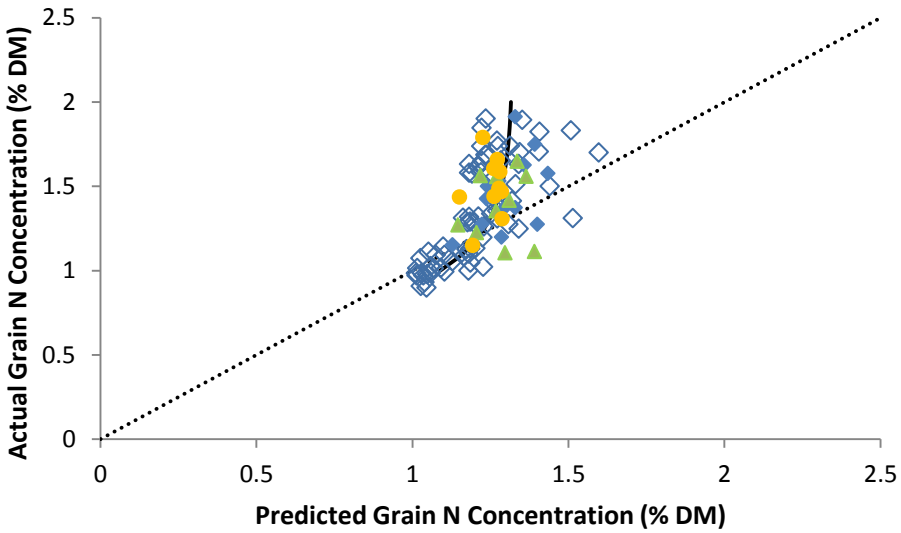

Figure 5.3. Relationship between actual and predicted grain N concentration calculated using canopy reflectance (NDRE) measurements at GS 59 as an explanatory variable RMSE= 0.294; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: 1.3214 – 9.76*(0.0238); R^2=0.48.

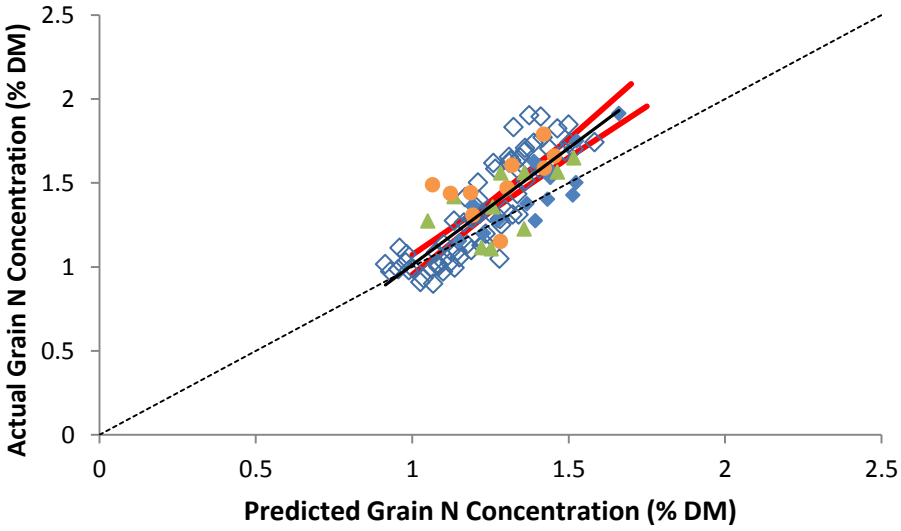

Figure 5.4. Relationship between actual and predicted grain N concentration calculated using leaf area index (LAI) measurements at GS 59 as an explanatory variable. RMSE= 0.246; broken line shows 1:1 relationship; (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.389x – 0.3766; R^2 = 0.66. ——— = Confidence Interval

Table 5.1. 95% Confidence and Prediction Intervals of the model using LAI, at a range of predicted grain N concentrations

		Predicted Grain N Concentration (% DM)				
Model	**Interval**	**1**	**1.25**	**1.5**	**1.75**	**2**
LAI	Confidence	0.059	0.031	0.055	0.099	0.146
	Prediction	0.321	0.317	0.320	0.331	0.348

5.3.2 Relationship of non-destructive measures of crop biomass and canopy N content after heading

5.3.2.1 Relationships between canopy N content, NDRE and indirect estimates of LAI

The relationship between NDRE and canopy N content (kg N ha^{-1}) at GS 59 for the three site-seasons is shown in Figure 5.5. A strong, curvilinear relationship ($P<0.001$) was found between GS 59 canopy N content and NDRE across sites, seasons and fertiliser N treatments, which accounted for a high level of variation ($R^2 = 0.97$). It spanned a wide range of crop N contents from 40 kg N ha^{-1} to 200 kg N ha^{-1}.

The relationship between LAI and GS 59 canopy N content (kg N ha^{-1}) is shown in Figure 5.6. Linear regression analysis of data pooled from the three site-seasons indicated that LAI explained only a modest level of the variation in canopy N content ($P<0.001$; $R^2 = 0.62$). However it was clear from visual observation of the data that the relationship between LAI and canopy N content differed between site-seasons. This was confirmed when a model that allowed site-season specific coefficients increased the proportion of variation explained to 90%. Thus two separate models were fitted to the data sets; (*a)* represents the Road Field experimental site with a significant linear relationship ($P<0.001$; $R^2 = 0.86$) and *(b)* represents the combined data from Bull Park and College Field ($R^2 = 0.91$).

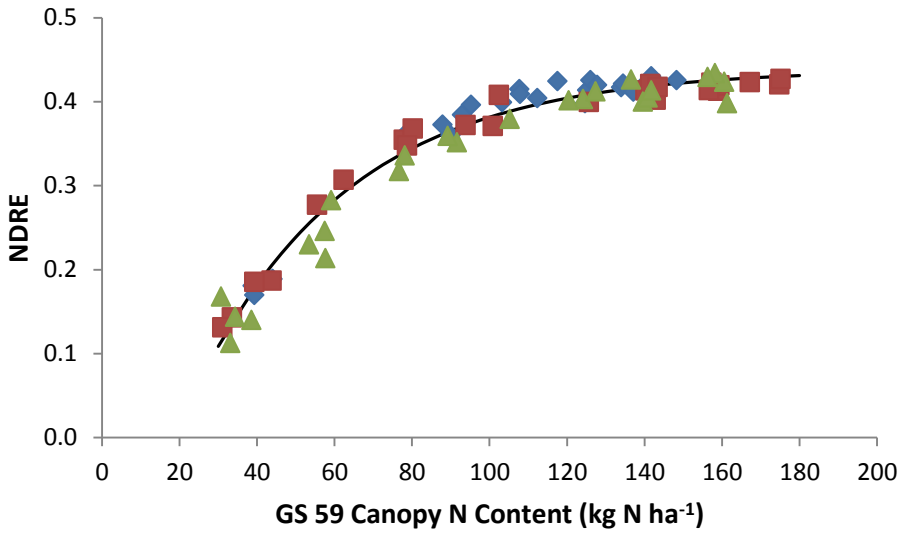

Figure 5.5: Relationship between NDRE and canopy N content (kg N ha^{-1}) at GS 59 ($P<0.001$; $R^2 = 0.97$). (◆) Road Field 2013, (■) Bull Park, (▲) College Field 2014. Each point represents an individual plot value.

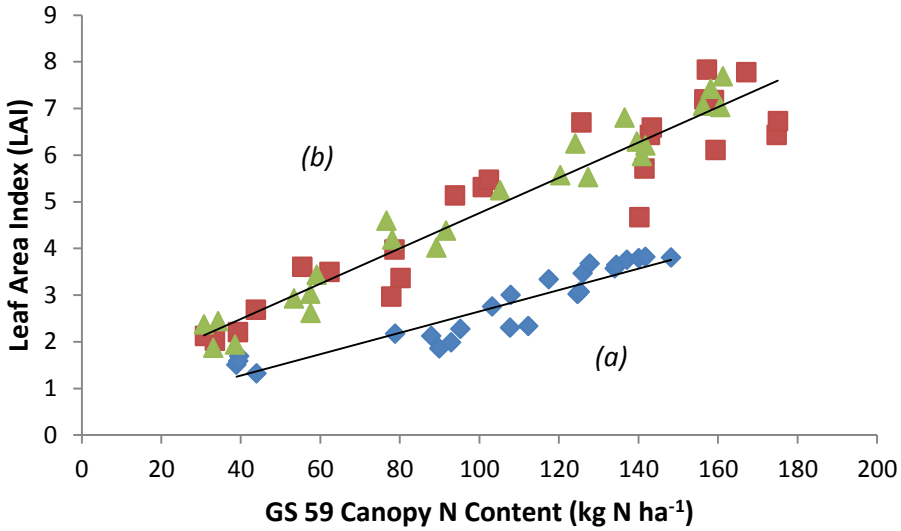

Figure 5.6: Relationship between leaf area index (LAI) and canopy N content (kg N ha^{-1}) at GS 59 (a) Road Field (2013) (◆) ($P<0.001$; $R^2 = 0.86$) and (b) combined Bull Park (■) and College Field (2014) (▲) data ($P<0.001$; $R^2 = 0.91$). Each point represents an individual plot value.

5.3.2.2 Relationships between crop biomass, NDRE and indirect estimates of LAI

Figure 5.7 illustrates the relationship between NDRE and GS 59 biomass. When data from the three site-seasons were combined a significant ($P<0.001$) linear relationship was found between NDRE and biomass at GS 59; however the level of variation explained was low ($R^2 = 0.46$). When the model was allowed to have separate site-season coefficients the percentage of variation explained increased to .89. There was a significant curvilinear relationship ($P<0.001$) between NDRE and aboveground biomass accumulation at GS 59 for the Road Field site that accounted 81% of variation in NDRE. When data were combined from Bull Park and College Field, GS 59 biomass accounted for 92% of the variation in NDRE ($P<0.001$). Additionally, there is some evidence that NDRE saturated at values of 0.4 which coincided with an aboveground biomass of around 10 t ha^{-1} at Bull Park and around 6 t ha^{-1} at Road Field.

A positive linear relationship ($P<0.001$) was found between LAI and GS 59 biomass which accounted for a good level of variation in GS 59 biomass ($R^2 = 0.73$) (Figure 5.8). The relationship was observed at the three sites-seasons when all data were combined. The relationship spanned a wide range of crop biomass levels from 4000 kg ha^{-1} to almost 12000 kg ha^{-1}.

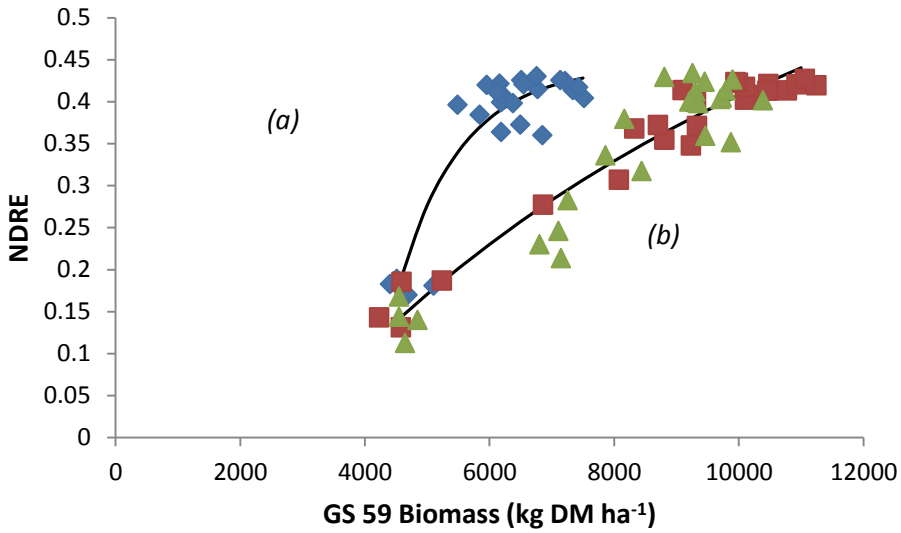

Figure 5.7: Relationship between NDRE and biomass (kg DM ha^{-1}) at GS 59 of *(a)* Road Field (2013) (◆) ($P<0.001$; $R^2 = 0.81$) and *(b)* combined Bull Park (■) and College Field (2014) (▲) data ($P<0.001$; $R^2 = 0.92$). Each point represents an individual plot value.

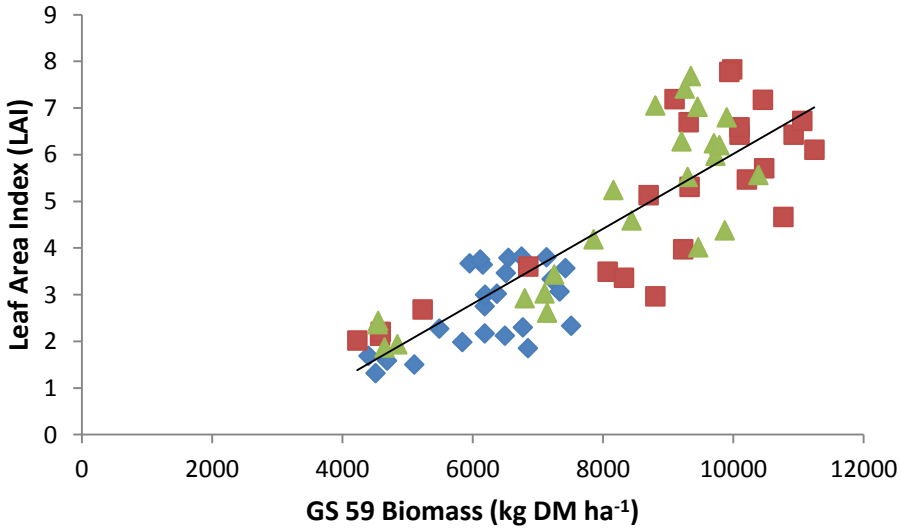

Figure 5.8: Relationship between leaf area index (LAI) and biomass (kg DM ha^{-1}) at GS 59 of combined Road Field (2013) (◆), Bull Park (■) and College Field (2014) (▲) data ($P<0.001$; $R^2 = 0.73$). Each point represents an individual plot.

5.3.3 Prediction of grain N concentration

As canopy reflectance (NDRE) displayed saturation with biomass and N content within the range experienced in this experiment, NDRE was deemed not suitable as a predictor of either variable. Therefore, LAI was used as a method to predict canopy N content and biomass at ear emergence. Estimates of crop N content and biomass at ear emergence were made for the validation crops in 2015 from measurements of LAI using the equations in Figures 5.6. (*b*) and 5.8 respectively. These were then used to predict grain N concentration with model 4b (Chapter 3). The relationship between actual and predicted grain N concentration (% DM) is presented in Figure 5.9. Over 66% of the variation in actual grain N concentration across sites, varieties and fertiliser N treatments was accounted for by the predicted N concentration and the RMSE was low (0.25). The relationship was exhibited across a range of grain N concentrations between 1 and 2%. However, the slope of the line was greater than 1 (1.46) indicating a tendency to over prediction at low N concentrations and under prediction at high N concentrations. The error appears to originate from the prediction of biomass (data not shown). Therefore, the model was repeated, using estimates of canopy N content derived from non-destructive measurements of LAI and actual biomass values acquired from destructive sampling (Figure 5.10). Predictions of grain N concentration were not as accurate or precise, as the level of variation accounted for was lower (59%) and the RMSE was larger (0.27), however the slope of the relationship between predicted and measured N concentration was close to 1.0, but there was a small under prediction across the range of N concentrations.

5.3.3.1 Confidence and Prediction intervals

The relationship using GS 59 N content and biomass estimates from LAI underestimated actual grain N concentration. The 95% confidence interval for the prediction was at ± 0.058 %N indicating that users can have a reasonable level of confidence in the predictions (Table 5.2). For example, with an average predicted grain N concentration of 1.5%, the user could be confident that the actual value at harvest would (with 95% probability) lie between 1.44 and 1.56%. However, the

model under predicted by an average of 0.2 %N. Calculated prediction intervals showed that the model was unsuitable for use as a predictor of grain N concentration using a single sample, because the margin of error of prediction was ± 0.32%.

The relationship using GS 59 N content obtained from LAI and actual biomass destructively sampled (Figure 5.10) also under estimated actual grain N concentration. The 95% confidence interval for the prediction was also ± 0.058 %N indicating that users can have a reasonable level of confidence in the predictions (Table 5.3). However, the underestimation was smaller than when biomass was estimated non-destructively. For example, with an average predicted grain N concentration of 1.5%, the user could be confident that the actual value at harvest would (with 95% probability) lie between 1.6 and 1.72%. However, the actual grain N concentration achieved would be 1.67% N, as a result of the models underestimation. The margin of error (0.35% N) associated with prediction using one sample was too large for practical applications.

Table 5.2. 95% Confidence and Prediction Intervals of predictions using N content and biomass obtained from LAI at a range of predicted grain N concentrations

	Predicted Grain N Concentration (% DM)				
Interval	**1**	**1.25**	**1.5**	**1.75**	**2**
Confidence	0.060	0.031	0.058	0.105	0.154
Prediction	0.321	0.317	0.321	0.333	0.351

Table 5.3. 95% Confidence and Prediction Intervals of predictions using N content obtained from LAI and actual biomass at a range of predicted grain N concentrations

	Predicted Grain N Concentration (% DM)				
Interval	**1**	**1.25**	**1.5**	**1.75**	**2**
Confidence	0.051	0.034	0.058	0.096	0.136
Prediction	0.352	0.350	0.353	0.362	0.374

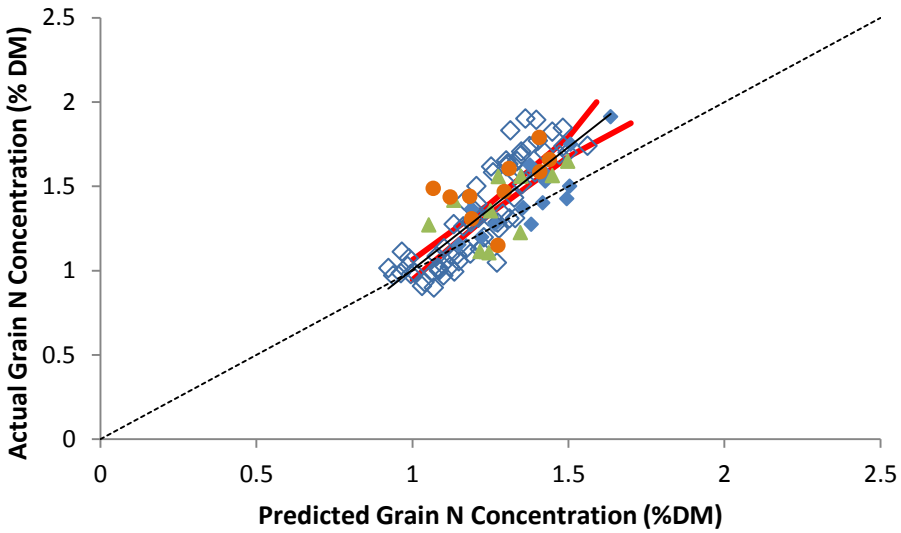

Figure 5.9: Relationship between actual and predicted grain N concentration calculated using surrogate values of canopy N content (kg N ha^{-1}) and aboveground biomass (kg DM ha^{-1}) obtained from leaf area index (LAI) measurements at GS 59. Predictions of grain N concentration were made using model 4 (b) developed from two sites in 2014, using canopy N content and aboveground biomass at GS 59 as explanatory variables. RMSE= 0.250; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.456x – 0.4507; R^2 = 0.66. ——— = Confidence Interval

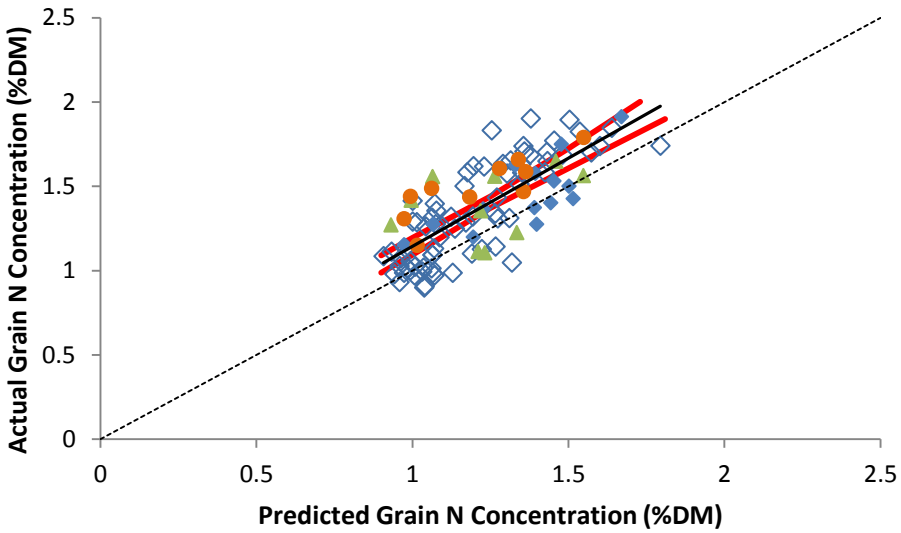

Figure 5.10: Relationship between actual and predicted grain N concentration calculated using surrogate value of canopy N content (kg N ha^{-1}) obtained from leaf area index (LAI) measurements and actual aboveground biomass (kg DM ha^{-1}) obtained from destructive sampling at GS 59. Predictions of grain N concentration were made using model 4 (b) developed from two sites in 2014, using canopy N content and aboveground biomass at GS 59 as explanatory variables. RMSE= 0.266; broken line shows 1:1 relationship. (◊) Taberna from experimental sites; (◆) Taberna, (▲) Propino, (●) Sanette, commercial crops. Slope of the line: y = 1.0443x – 0.996; R^2 = 0.59. ——— = Confidence Interval

5.3.4 **N partitioning within the canopy and saturation of NDRE**

Figure 5.11 shows the relationship between the partitioning of N to individual ear and leaf layers at GS 59 (kg N ha^{-1}) and total canopy N content (kg N ha^{-1}). As the canopy N content increased in the crop, the N content increased linearly in leaf layer 1, 3, 4 and 5+ across the range of canopy N contents from approximately 25 to 150 kg N ha^{-1}. However, the rate of increase declined in the ear and increased in leaf layer 2 at 100 kg N ha^{-1}. This indicates that as the N content of the canopy increased above 100 kg ha^{-1} relatively more of the additional N was allocated to leaf layer 2 and less to the ear. Thus there was a shift in the relative partitioning of N to leaf layer 2 when the canopy N content increased above 100 kg N ha^{-1}.

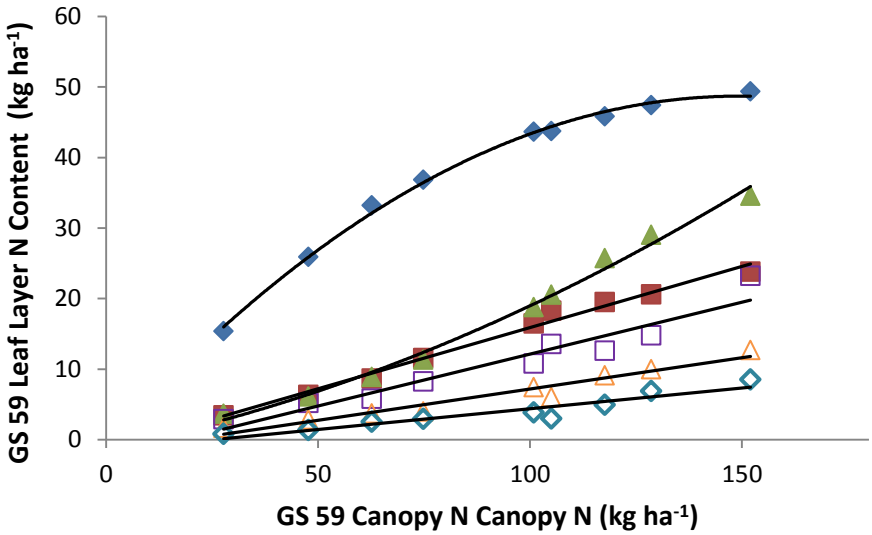

Figure 5.11: Relationship between total canopy N content and N contents of ear and leaf layers (leaf lamina, true stem and leaf sheath). Ear (◆) ($P<0.001$; $R^2 = 0.92$), leaf 1 (■), ($P<0.001$; $R^2 = 0.96$), leaf 2 (▲) ($P<0.001$; $R^2 = 0.97$) leaf 3 (□) ($P<0.001$; $R^2 = 0.83$), leaf 4 (Δ) ($P<0.001$; $R^2 = 0.87$), leaf 5+ (◊) ($P<0.001$; $R^2 = 0.70$). Leaf layers are numbered from the top of the canopy downwards where the flag leaf is leaf 1. Each point represents mean plot values of four replicates.

Figure 5.12 shows a strong, positive relationship found between SPAD measures of individual leaves and total canopy N content at ear emergence (kg N ha^{-1}). The relationship was observed from a range of N contents of 25 kg N ha^{-1} to 160 kg N ha^{-1}. For each leaf, linear regressions fitted the data well and there was no evidence of saturation in SPAD values at high canopy N contents. With leaf 1 there was an indication of a possible change in slope above canopy N contents of 70 kg N ha^{-1}.

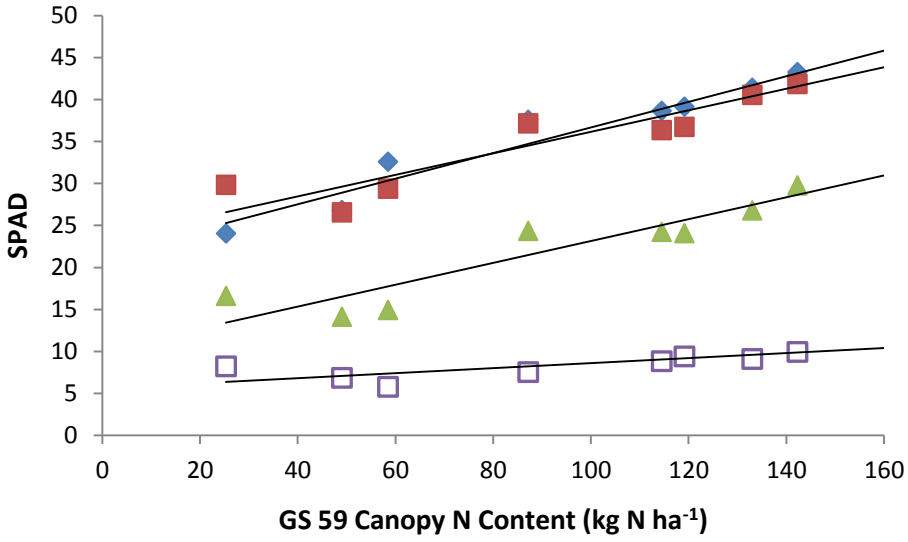

Figure 5.12: Relationship between SPAD measurements of individual leaves and total canopy N content (kg N ha^{-1}) at GS 59 for leaf 1 (flag leaf) (◆) ($P<0.001$; R^2 = 0.84), leaf 2 (■) ($P<0.001$; R^2 = 0.70), leaf 3 (▲) ($P<0.001$; R^2 = 0.52), leaf 4 (□) ($P=0.047$; R^2 = 0.09). Symbols represent treatment means of 4 replicates.

5.4 **Discussion**

Malting barley markets require grain N concentration of 1.52-1.84% (Pettersson, 2007). Producers that achieve malting barley grain N concentration specifications receive a price premium over those of lower value markets. However, variation in grain N concentration can be caused by environmental conditions (rainfall and temperature) and agronomic practices such as fertiliser N applications. Prediction models of grain N concentration were developed in Chapter 3 and validated on an independent data set in Chapter 4, using measures of N content and biomass at ear emergence. Non-destructive measurements such as indirect measures of LAI and canopy spectral reflectance can provide rapid, cost-effective and non-invasive estimates of such crop variables (Montes *et al.*, 2007). Additionally, such non-destructive measurements allow a greater number of measurements to be taken and in a shorter period of time compared to destructive methods, ultimately increasing the possible sample area (Pittman, *et al*., 2015).

Non-destructive measurements of NDRE and LAI at ear emergence were related to grain N concentration as in Figures 5.1 and 5.2 respectively. There were distinct differences in the relationships between years due to water stress experienced in 2013 as described in Chapter 3. The combined data for Bull Park and College Field was used to predict grain N concentration using the validation data in 2015. Although NDRE and LAI were comparable in the variation explained of grain N concentration, NDRE produced a curvilinear relationship, which eliminated it as a possible prediction model.

NDRE was strongly related to canopy N content at ear emergence across sites and years, even when the crop growth was limited by drought conditions, as shown in Figure 5.5, suggesting it may be a potentially valuable method of non-destructively estimating canopy N content. NDRE was strongly related to aboveground biomass at GS 59, although two distinct relationships were evident due to differences in biomass production between years as a result of pre-anthesis moisture stress and subsequent reduction in canopy expansion and solar radiation interception. However, the

vegetation index used in this study displayed a distinct saturation effect when at higher values of both canopy N content and biomass.

Canopy reflectance measures the absorption of light from visible (VIS) wavelengths by leaf chlorophyll and associated pigments. There is a low level of reflectance in the visible wavelength as chlorophyll absorbs a high level of light energy. The near infra-red (NIR) wavelengths have high level of reflectance as a result of different leaf tissues (Knipling, 1970). Several experiments have been conducted using canopy spectral reflectance to measure biomass (Chen *et al*., 2009; Sellers, 1987), grain yield (Li-Hong *et al*., 2007; Aparicio *et al*., 2000), total N uptake (Stone *et al*., 1996), green leaf area (Best and Harlan, 1985) and N content (Wright *et al*., 2004).

Canopy reflectance measurements of both canopy N content and biomass at ear emergence showed a distinct saturation effect when reflectance sensor values reached 0.4, which coincided with N contents of approximately 100 kg N ha^{-1} over both experimental years and biomass levels of 6 t DM ha^{-1} and 10 t DM ha^{-1} in 2013 and 2014 respectively. Therefore accurate estimates of variables will be difficult to achieve (Tucker 1977, Gitelson *et al*., 1996). The optical properties of vegetation canopies vary greatly over time due to changing plant status, LAI, fractional vegetation cover and phonological life form. As a plant's canopy develops, the impact of soil reflectance gradually decreases and is replaced by the developing plant spectra of leaf. As the green vegetation gets denser, the spectral reflectance saturates. However, the LAI at which saturation occurs varies as a result of spectral region or wavelength. As there is low penetration depth in the visible wavelength from the top of the canopy saturation occurs at an LAI of approximately 1-2. Conversely, NIR wavelengths are capable of penetrating deep into the canopy, resulting in an NIR enhancement effect in which much of the NIR energy scattered and transmitted from upper leaves can be reflected by lower leaves and retransmitted from upper leaves to enhance reflectance (Asner, 1998). The saturation level for NIR is reached around an LAI of 5 or 6. The value of LAI in respect to canopy reflectance saturation is also influenced by plant geometry. The value of LAI corresponding to saturation will also vary with plant geometry as greater LAI values are required to reach saturation

points in plants that possess erect leaves compared to those with horizontal leaves (Guyot, 1990).

Estimates of LAI obtained through SunScan measurements (intercepted photosynthetically active radiation (PAR)) related in a strong linear fashion to canopy N content at ear emergence up to almost 200 kg N ha^{-1}. However, the relationship was not robust across years as displayed in Figure 5.6. This can be explained by the restriction in canopy growth due to pre-anthesis drought which was experienced in 2013. However, despite reduced canopy size, canopy N content for Road Field (2013) was comparable with those of the two sites in 2014. This is further illustrated in Table 3.1 in Chapter 3, showing canopy N concentrations and NNI. However, the relationship was influenced due to restricted canopy size and unaffected N uptake within the canopy. A strong robust linear relationship was exhibited between GS 59 biomass (kg DM ha^{-1}) and LAI (Figure 5.8). The relationship was observed at all three site-seasons of experiments when all data were combined. Monteith (1972, 1977) observed that a canopy's net primary production is proportional to the canopy's intercepted solar radiation. Therefore biomass production can be related as a linear function of intercepted photosynthetically active radiation (PAR). This linear relationship occurs despite the curvilinear relationship (and saturation) evident between PAR and photosynthesis of leaves at canopy level. An explanation of this could be due to the N distribution of leaves within the canopy in the form of the light gradient, which maximizes canopy photosynthesis (Hirose and Werger, 1987) in a linear relationship with incident PAR (Kull and Jarvis, 1995; Charles-Edwards, 1982).

The use of non-destructive methods to estimate canopy N content and biomass produced a good prediction of grain N concentration. When actual observed values were plotted against predicted concentrations, a lower percentage of variation was accounted for (66%) compared to 80% when direct measures were used in the model 4b. However, as the relationship was not equal to one, it was investigated and found that the error of prediction may originate from biomass estimation. As the principles of an ideal prediction model require easy to use parameters and cheap operation, non-destructive estimates of N content obtained from LAI and actual biomass values

were used in model 4b (Figure 5.10). Although the R^2 was lower at 0.59, the slope of the line was closer to one and the RMSE was only marginally larger, but under estimated grain N concentration by only 0.16%.

Nitrogen concentrations in the individual plant and crop canopies are not uniform, due to differing levels of light intensity due to factors such as shading by upper leaves (Aerts and De Caluwe, 1994; Gastal and Lemaire, 2002). The N content in the canopy increases from the bottom to the top in a pattern that mirrors light distribution within the canopy (Hirose and Werger, 1987; Hirose *et al*., 1989). Furthermore, as a result of the reduction in irradiance levels within the canopy, lower leaves require less nitrogen for optimum carbon assimilation (Gastal and Lemaire, 2002). Hirose and Werger (1987) found that plant canopies that displayed the greatest nitrogen content per unit area, in the regions of the most irradiation, demonstrated an advantage in terms of their photosynthetic capacity over canopies with uniform nitrogen distribution throughout. This conforms to the suggestion of Field (1983), that photosynthesis would be maximised by distributing nitrogen to canopy regions receiving the greatest levels of irradiance. Crops have been shown to be close to the optimal distribution of leaf N in terms of maximising carbon gain (Werger and Hirose, 1991). The findings presented suggest that as N content in the canopy increases above 100 kg N ha^{-1}, more N was stored in the leaf layers 2, in comparison to the top leaf layers. This would correspond with the saturation experienced with canopy reflection. Although not tested directly, it may be a contributing factor to the saturation issues associated with canopy reflectance and its relationship with canopy N content.

SPAD measurements showed good correlations with canopy N content and did not suffer from the same saturation affects at high N contents as canopy reflectance. In comparison to canopy reflectance measures, SPAD measurements operate on the basis of absorbance of two wavelengths, the red light (650nm) is absorbed by chlorophyll and infrared light (940 nm) which is not absorbed by chlorophyll (Xiong *et al*., 2015). Majority of the nitrogen found in leaves is located in chlorophyll; therefore, quantifying chlorophyll content would allow an indirect estimation of the N status of a crop (Filella, *et al*., 1995). Chlorophyll meters have been used by others

to estimate foliar Chl and N content in wheat (Le Bail *et al.,* 2005). Martinez and Guiamet, (2004) found that chlorophyll meter measurements could be distorted by a number of factors including a change in irradiance levels, water content and diurnal changes (although more in lower chlorophyll content plants such as those 0 N fertiliser treatments). The authors suggested that, as such changes in SPAD results could differentiate between N surplus and N deficiency that such factors need to be considered when using such a non-destructive method. Hamblin *et al. (*2014) found that variety can have large influences on SPAD results, which may require cultivar specific models to be produced. However, the SPAD results are preliminary findings and were not validated in this study. But it could provide a promising alternative in comparison to canopy reflection and the associated limitations due to saturation influences.

Both forms of non-destructive measurements offer advantages and disadvantages. Canopy reflectance provided a robust relationship with canopy N content at ear emergence; however, limitations due to vegetation index saturation at values of 0.4 were evident. Indirect measures of LAI produced linear relationships with N content and biomass, but these relationships were affected by year. The use of non-destructive methods to estimate N content and biomass at ear emergence can provide rapid and non-invasive estimates of crop variables, which offers the ability take a larger number of samples. However, this advantage comes at a loss of accuracy when compared to direct measures.

From the results of N partitioning within the canopy across a wide range on N treatments, it can be suggested that as N content increases, the distribution of N is altered and thus may contribute to the saturation effect the relationship between N content and canopy reflectance. However, a promising alternative to canopy reflectance may be the chlorophyll meter. Although only initial findings, it was not affected by saturation as was canopy reflectance at high canopy N contents. But results must be used tentatively as environmental factors and variety can distort results.

6 General Discussion and Conclusions

The research reported in this thesis used a multiple regression approach to produce statistical models for predicting grain N concentration of barley from crop measurements made at ear emergence. Variables suitable for inclusion in the models were identified from the strength of their relationship with the components of grain N concentration and their consistency across sites and seasons. The best model was based on variables that related well to grain N content and yield, namely canopy N content and biomass at ear emergence and gave a good accuracy of prediction with R^2 of 0.8 and RMSE of 0.22% N when validated with independent validation data. The model could, therefore, be a valuable tool to aid crop management decisions by growers and post-harvest operations by maltsters. In this final chapter, the practical aspects, the benefits and the potential limitations of using predictions of grain N concentration are considered.

The precision of the predictions (R^2=0.8) using the best model in this project was considerably better than those that have been reported previously. For example Le Bail *et al. (*2005) reported an R^2 of 0.52 for the relationship between grain N concentration of wheat predicted at GS65 and actual grain N concentration. However, in the current study the relationship overestimated actual grain N concentration slightly and thus the prediction needs to be corrected before it can be used in practice.

Nevertheless, the 95% confidence interval for the prediction was narrow at ± 0.035 % N indicating that users can have a high level of confidence in the predictions. For example, with an average predicted grain N concentration of 1.5%, the user could be confident that the actual value at harvest would (with 95% probability) lie between 1.465 and 1.535% N. Calculated prediction intervals showed that the model was unsuitable for use as a predictor of grain N concentration using a single sample, because the margin of error of prediction was ± 0.35%. This margin of error is too large for commercial purposes as possible grain N concentrations that might arise from a predicted value of 1.5% span the range acceptable for malting. Thus, as long as single samples are not used as the basis for prediction, Model 4b presents a

feasible option for producers and or maltsters to achieve an accurate estimate of grain N concentration. It can be suggested that an average of approximately 5 or more samples would give an accurate estimate of grain N concentration for a given field, farm or region. However, the number of samples required to provide an accurate estimation of grain N concentration would also be dependent on the scale of within-field variation in crop growth. The greater the variation, the larger the number of samples that will be required for an accurate prediction of the average grain N concentration for the field as a whole.

If the forecasting system is to be taken up and used in practice, the measurements required need to be relatively quick and inexpensive for users to make. This may require some trade-off between ease of measurement, cost and accuracy of prediction. Soil mineral N content generally did not contribute much to the variation of grain N concentration, with the exception of the models including 2013 data, where high soil mineral N content was experienced. From a measurement perspective, this is advantageous, as determination of soil mineral N content is a laborious task involving specialist equipment, time and additional cost. Compared to measurements of crop biomass, the calculation of potential grain sites is relatively quick and easy for producers to carry out and can be repeated at a number of locations to achieve an accurate estimation. However, the proportion of variation in grain N concentration that was accounted for by including potential grain sites in the model was never greater than that accounted for by biomass, and when both were combined, added little to the model. However, destructive sampling for determination of crop biomass and N content may present growers with difficulties. Producers could sample a known area of crop and send samples to a suitably equipped laboratory for analysis, but the storage of samples prior to and during transport and the time involved in analysis may have implications. If samples are not dried on farm, fresh samples will need to be dispatched rapidly in chilled containers to minimise changes in tissue composition prior to analysis. Delays in analysis and communication of results to growers could impact on management decisions and reduce opportunities for applying remedial N on crops at risk of low grain N concentration. This raises questions as to the possibility of predicting grain N concentration at an earlier stage of growth, allowing more time for an informed

response from the producer. However, evidence suggests that predictions made at earlier growth stages are likely to be less accurate than those made later. Accuracy of prediction of grain N concentration in wheat was increased when predictions were based on measurements made at GS 71 compared to GS 65; R^2 increased by over 10% from 0.64 to 0.77 (Le Bail *et al*., 2005). It has been suggested that this is because the later timing allows more of the residual fertiliser N to be taken up prior to measurement. This is less likely to be an influence in barley because unlike wheat that is grown for high grain N, fertiliser recommendations for malting barley are lower and the majority of fertiliser N is taken up before flowering as shown for 2014 in Chapter 3 (Figure 3.7.). Similar results were shown by Weightman *et al. (*2011), where prediction of grain N concentration using samples taken at the milky ripe stage were better indictors than samples taken at anthesis. Although not investigated in this study, earlier predictions at GS39 for example could prove to be less accurate than those made at ear emergence. Thus any benefits gained in terms of timelines of operations from an earlier prediction of grain N concentration must be weighed against a possible loss of accuracy.

Non-destructive methods offer the potential to rapidly (almost instantaneously) predict grain N concentration removing the concern of slow turnaround and maximise effectiveness of any remedial actions should they be deemed appropriate. Non-destructive measurement allows a much greater sample size to be collected in comparison to destructive methods thereby reducing the effect of small scale spatial variation. Non-destructive measurements would also reduce costs to a fraction of the cost per unit compared to destructive sampling.

However, in the current study, the non-destructive methods were not as accurate as destructive methods in the prediction of grain N concentration, although, the potential to attain a greater number of samples may compensate for the lower accuracy associated with such methods. Developments in non-destructive sensor technology may reduce the limitations that are experienced regarding saturation at high canopy biomass and N content. If this could be achieved it would be a major step forward because reflectance methods provide a robust measure of variables up to the saturation point across sites and seasons. The ability to conduct these

measurements via tractor mounted instruments in association with other husbandry practices such as fungicide applications at ear emergence would reduce labour requirements. Additionally, the almost instant prediction would allow timely decisions to be made regarding late season N fertiliser to maximise N uptake and return on investment by achieving malting specification.

Maltsters would be inclined to use the prediction model in a more strategic approach with the emphasis of forecasting for post-harvest volume and logistics planning. Samples would be taken on a regional basis to estimate seasonal expectations of grain N concentration based on geographical location. Although maltsters are likely to prefer non-destructive measurement methods to keep costs down, they might be more willing and able than growers to bear cost of destructive techniques if the predictions are more reliable. Moreover, the use of predictions for strategic purposes is less constrained by time as there would not be the same urgency to get results back to make decisions about application of fertiliser. On the other hand, if widespread below specification grain N concentrations are forecasted, maltsters may wish to adopt a more tactical approach and recommend remedial actions to be taken by producers. In this case fast turnaround would again be important. As accurate predictions from destructive sampling benefit maltsters as a means to maximise the consistent supply of satisfactory grain N concentration requirements, the cost of such a process may be absorbed by the maltsters or possibly through the payment of larger premiums to growers.

The validation experiment examined the effect of season on the robustness of the prediction model by using the malting barley variety Taberna (Quench x Taphouse), as used in the model development. In addition to this, two other varieties were selected to assess the effect of variety on the model's performance. Another malting variety Propino (Quench x NFC Tipple) was selected to be included. Both Taberna and Propino share a common lineage through the variety Quench. Sanette (Sumit x Yard) was chosen as a third variety as it is not related to Taberna or Propino, and is not a recommended malting barley variety and is grown for feed markets. Although the model predicted well using data from these three varieties, it cannot be assumed that all varieties would behave similarly. Accounting for variety as a factor in

predictions of grain N in wheat significantly improved the accuracy of predictions (Le Bail *et al.,* 2005). There was no clear association with group of the variety for end use and in their models the variety effect was believed to result from differences in the relationship between SPAD readings and leaf N concentration rather than the dynamics of N uptake and partitioning to grain. In barley this source of error between varieties may not be a problem if direct measurements of canopy N are made. However, varieties might be expected to differ in grain N deposition and grain size (TGW). Data acquired from Irish and UK Recommended Lists (Anon 2014a; Anon 2014b) illustrates variation in TGW and grain N concentration of varieties from 45.8 – 49.8g and 1.42 – 1.53 % respectively. However it should be noted that these values are for varieties specifically selected for malting market requirements. If feed market varieties are included, the level of variation may be increased. Validation of predictions over a wider range of varieties under different growing conditions is needed if the industry is to have confidence in the predictions. The ability of the model to perform satisfactorily over a larger number of varieties remains to be evaluated. Varieties that display different characteristics to those used in the development of the model may impact on the relationship's accuracy. Varieties that recover a larger or smaller fraction of N from the canopy and transfer it to the grain (resulting in a larger or smaller NHI) or those with a different post anthesis N uptake (PANU) would result in a different grain N content than would be expected using the current prediction model. Likewise, variation between cultivars in their carbohydrate loading would also influence the relationship. As the assimilate for grain fill is sourced from pre-anthesis dry mater accumulation and post anthesis carbon assimilation (Sanford and MacKown, 1987), differences in the crop's resource use efficiency and dry matter allocation could significantly impact on grain yield and the models performance. Although new varieties may be reaching the upper limit of harvest index (HI) of 0.5-0.6 (Hay, 1995; Miralles and Slafer, 2007), variation in HI between cultivars exists and genetic differences would impact the relationship and ultimately the prediction. It may be necessary to produce variety specific prediction models due to differences in their grain filling process.

It is worth noting that the validation included a number of crops outside of the normal commercial range of fertiliser N application rates, from the N response

experiments. Although these rates are unlikely to be used in practice, they were included in the validation to test the models' robustness to predict across a wide range of crop nutritional status and size.

Models produced in 2014 predicted well in 2015, but the weather conditions in the two seasons were similar. It is clear that the relationships between crop growth characteristics that underlie the predictions can vary between seasons. In 2013 where low rainfall and shallow soil created conditions of crop water stress, canopy growth from GS 39 was restricted and the result was high grain N concentrations. The relationship between crop biomass measurements at ear emergence and yield were more sensitive to seasonal effects than the relationship between canopy N content at ear emergence and harvest. It was argued in Chapter 4 that the weather conditions experienced in 2013 are very unusual for Ireland. However, currently it is not known how far the weather conditions could deviate from the long term average before the accuracy of the predictions is reduced. Wider testing across years will be needed to determine this. Should a loss of accuracy be found when a larger number of seasons are investigated, the next question will be what can be done about it. There are a couple of approaches that might be taken. The first is to develop separate models (or refine the existing model) for use with different weather patterns. This will require the collection of a lot more data and take a considerable amount of time to ensure a wide enough range of weather conditions are covered between regions and/or across years. The second approach would be to identify crops and years where the current model(s) are unlikely to be accurate. Here expert judgement can then be used to assess whether the grain N concentration will be greater or lower than that predicted. Since adverse conditions during late stem extension and subsequent grain filling are likely to reduce yield formation more than grain N accumulation, under prediction of N concentration is more likely to occur than over-prediction. In 2013 crops had an unusually small canopy (LAI) and biomass, but relatively large N content. A combination of measurements such as LAI, NDVI and possibly SPAD could be used to identify these situations and highlight crops where the prediction may prove unreliable. Meteorological data on rainfall, temperature and potential soil moisture deficit could also help inform growers as to when the prediction might lose accuracy. In terms of the effect of season, it may be proactive to continue to collect samples

yearly to observe the relationship. If a long term change in weather conditions occurs, rather than extremes in terms of seasonal fluctuations, an equation developed as a rolling average over the previous number of years would accommodate such changes. This would provide a greater level of confidence in the prediction model.

The second objective investigated the effects of applications of anthesis fertiliser N on N uptake and grain N concentrations in crops of various canopy N content prior to grain filling. Anthesis N increased grain N concentration by 0.2 and 0.3% with the application of 30 and 40 kg N ha^{-1} respectively. This would mean that producers with crops predicted to be at risk of low grain N concentration can use the information in a tactical approach. Crops predicted to be just below the threshold for malting markets would receive additional N at anthesis to ensure that crops reach market grain N concentration requirements and price premiums are achieved. Ireland's cool temperate oceanic climate may put it at a distinct advantage in terms of recovery of late applied N fertiliser. The recovery efficiencies of over 50% grain N on average in favourable years would encourage the use of late season fertiliser to rectify crops at risk of low grain N concentration. However, this does not guarantee the crop will satisfy other malting barley market requirements, which include moisture content, germination capacity and screenings (Spink *et al.,* 2014). Producers should try to capitalise on crop husbandry practices such as late season ear sprays for example, to maximise quality and achieve contract specifications and receive price premiums.

In conclusion the work presented in this thesis has shown that grain N concentration can be predicted with good accuracy from measurements of canopy N content and biomass at ear emergence under conditions similar to those encountered in the 2014 and 2015 seasons. Destructive measurements provide the greatest accuracy, but non-destructive measurements of LAI and canopy reflectance also provide a reasonable prediction. The prediction model is a potentially valuable tool for use by producers to predict the likely grain N concentration at harvest and facilitate decisions about the likely benefit of additional fertiliser N applications. It can also be used as an aid to schedule harvesting such that crops likely to satisfy the grain nitrogen concentration specification for malting, and hence attract a price premium, are harvested before lower value crops. It is also of potential value to maltsters as a method to forecast

regional and national volumes of grain with the required grain N concentration pre-harvest. Further testing of the accuracy of predictions across a wider range of sites, seasons and varieties will give the end user greater confidence in the value of the forecasting system.

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Appendix 1. Experimental site details of operations and P, K and Mn fertiliser and pesticide applications

Treatment	Road Field
Sowing Date	**26/03/2013**
Kieserite	**02/05/2013** 100 kg ha^{-1}
Herbicide	**10/05/2013** IPU 1 l ha^{-1}
	20/05/2013 Cameo Max 25 g ha^{-1} Duplosan 1 l ha^{-1}
Insecticide	**20/05/2013** Sumi-Alpha 165 ml ha^{-1}
PK Fertiliser	**01/03/2013** 0:7:30 100 kg ha^{-1}
	17/05/2013 80 kg KCl ha^{-1}
Nitrogen	**14/05/2013** **07/06/2013**
Fungicide	**22/05/2013** Fandango 1 l ha^{-1} **20/06/2013** Siltra 1 l ha^{-1} Bravo 1 l ha^{-1}
Trace Element	**22/05/2013** Multimin 1.5 l ha^{-1}
Harvest	**13/08/2013**

Treatment	Site	
Sowing Date	**College Field** **10/04/2014**	**Bull Park** **22/04/2014**
Kieserite	**01/05/2014** 100 kg ha^{-1}	**01/05/2014** 100 kg ha^{-1}
Herbicide	**15/05/2014** Inca 25g ha^{-1} Duplosan 1 l ha^{-1}	**15/05/2014** Inca 25g ha^{-1}
Insecticide	**15/05/2014** Sumi-Alpha 165 ml ha^{-1}	**15/05/2014** Sumi-Alpha 165 ml ha^{-1}
PK Fertiliser	**12/03/2014** 00:07:30 250 kg ha^{-1}	**22/04/2014** 00:07:30 395 kg ha^{-1} **09/05/2014** KCl 125 mg ha^{-1}
Nitrogen	**13/05/2014** **27/06/2014**	**20/05/2014** **03/07/2014**
Fungicide	**15/05/2014** Fandango 1 l ha^{-1} Mobius 0.75 l ha^{-1} **13/06/2014** Siltra 0.5 l ha^{-1} Bravo 0.5 l ha^{-1} **26/06/2014** Siltra 1 l ha^{-1} Bravo 1 l ha^{-1}	**15/05/2014** Fandango 1 l ha^{-1} Mobius 0.75 l ha^{-1} **27/06/2014** Siltra 0.5 l ha^{-1} Proline 0.4 l ha^{-1} Bravo 0.5 l ha^{-1}
Trace Element	**13/05/2014** Amazinc 1 l ha^{-1} Headland manganese 0.5 l ha^{-1} Barclays Zinc EDTA 0.25 l ha^{-1}	**15/05/2014** Mancozin 1 l ha^{-1}
Harvest	**13/08/2014**	**18/08/2014**

Appendix 2: Compiled weather data for experimental growing seasons 2013, 2014 and 2015 along with 30 year long term averages (LTA) 1981- 2010.

Year	Factor	Mar	Apr	May	June	Jul	Aug
2013	Max Temperature ^{o}C	11.9	17.4	19.3	23.4	29.5	24.3
	Min Temperature ^{o}C	-3.4	-3.8	1.8	5.3	7.4	5.9
	Mean Temperature ^{o}C	3.8	7.5	10.4	13.7	18.2	16.3
	Rainfall (mm)	57.6	44.4	35.6	37.5	32.3	85.6
2014	Max Temperature ^{o}C	15.1	18.2	20.9	24.9	27.6	22.4
	Min Temperature ^{o}C	-1.9	1.2	5.2	5.3	7.2	5.7
	Mean Temperature ^{o}C	7	10.1	11.9	14.5	16.9	14.5
	Rainfall (mm)	65	52.6	78.6	61.9	24.6	122.1
2015	Max Temperature ^{o}C	14.2	18.9	19.2	24	23.4	23
	Min Temperature ^{o}C	-2.2	-0.4	1.6	3.6	6.4	6
	Mean Temperature ^{o}C	6.2	8.7	10.2	13.4	14.6	14.6
	Rainfall (mm)	53.5	26.3	89.4	29.7	79.4	83
	LTA Rainfall (mm)	63.4	55.9	59.8	60.8	58.7	71.9
	LTA Temperature ^{o}C	6.9	8.4	11	13.7	15.6	15.3

Appendix 3: Description of site location and crop husbandry details for commercial crops used in 2015 validation experiment

Location	Variety	N Rate (kg N ha^{-1})
Wexford	Taberna	130
Wexford	Taberna	130
Wexford	Propino	130
Wexford	Propino	130
Wexford	Sannette	150
Wexford	Sannette	150
Cork	Taberna	150
Cork	Taberna	150
Cork	Taberna	140
Waterford	Sannette	140
Waterford	Sannette	140
Waterford	Taberna	140
Waterford	Taberna	150
Waterford	Taberna	150
Tipperary	Sannette	150
Tipperary	Sannette	150
Tipperary	Sannette	150
Tipperary	Propino	80
Tipperary	Propino	80
Cork	Taberna	80
Cork	Taberna	140
Cork	Propino	140
Cork	Propino	140
Cork	Propino	170
Laois	Taberna	170
Laois	Taberna	170
Laois	Taberna	170
Laois	Taberna	170
Laois	Sannette	130
Louth	Sannette	130
Louth	Sannette	130
Louth	Propino	150
Louth	Propino	150
Louth	Propino	150

Commercial crops were managed for maximum yield and quality, while adhering to maximum permitted fertiliser limits and treated with pesticides (herbicides, fungicides and insecticides) based on professional agronomic advice.

Appendix 4: Legislation regarding nitrogen fertiliser use on Irish farms (Wall and Plunkett., 2016).

In order to simplify advice tables, it is normal to classify soil available levels of major and micro nutrients into classes. The class is referred to as the Soil Index. At Johnstown Castle, soil analysis levels are classified into Index levels 1 – 4. The exact interpretation of the Soil Index varies somewhat with the element and the crop but the definitions in Table 1 apply in most circumstances.

Table 1: The Soil Index System

Soil Index	Index Description	Response to Fertilisers
1	Very low	Definite
2	Low	Likely
3	Medium / Adequate	Unlikely / Tenuous
4	Sufficient / High	None

The Soil N Index takes into account the past farm management history and reflects the likely rate of release of N from the soil.

Table 2: N Index for tillage crops that follow short leys or tillage. This table can also be used for grass establishment.

Index 1	Index 2	Index 3	Index 4
	Previous Crop		
Cereals, Maize	Sugar beet, Fodder beet, Potatoes, Mangels, Kale, Oil seed rape Peas, Beans, Leys (1 - 4 years) grazed or cut and grazed. Swedes removed.	Swedes grazed in situ	
Vegetables receiving less than 200 kg/ha N.	Vegetables receiving more than 200 kg/ha N.		

In continuous tillage it is usually only necessary to consider the last crop grown to estimate N. Index **(Table 2).** However, where long leys or permanent pasture occur in the rotation, it is necessary to consider the field history for longer than one year **(Table 3**). Previous applications of animal manures must also be taken into account.

Table 3: N Index for pasture establishment or tillage crops that follow long leys (5 years or more) or permanent pasture

Index 1	Index 2	Index 3	Index 4
		Previous Crop	
Any crop sown as the 5th tillage crop following long leys or permanent pasture. For subsequent tillage crops use Table 6-2.	Any crop sown as the 3rd or 4th tillage crop following long leys or permanent pasture. If original long ley or permanent pasture was cut only use Index 1.	Any crop sown as the 1st or 2nd tillage crop following long leys or permanent pasture (see also Index 4). If original long ley or permanent pasture was cut only use Index 2.	Any crop sown as the 1st or 2nd tillage crop following very good long leys or permanent pasture which was grazed only.

Table 4: Available N[1] for barley (kg/ha) for moderate yields[2] or where proof of higher yields is not available

Soil N Index[3]	Winter Barley[4]	Spring Barley[4,5,6]
1	180	135
2	155	100
3	120	75
4	80	40

1. N rates shown above refer to application rates of available fertilizer. Chemical fertilizer rates should be calculated by deducting the available N contained in organic fertilizer applications from the rates shown in the above table.

2. Winter Barley up to 8.5 tonnes/ha, Spring Barley up to 6.5 tonnes/ha.

3. See Tables 6-2 and 6-3 for Soil N Index.

4. Where proof of higher yields is available, an additional 20kg/ha N may be applied for every 1 tonne above reference yield see table 16-4. The higher yields shall be based on the best yield achieved in any of the three previous harvests at 20% moisture content.

5. To reduce the risk of poor establishment in spring cereals, not more than 75 kg/ha N should be combined drilled.

6. Where malting barley is grown under a contract to a purchaser of malting barley an extra 20 kg/ha N may be applied where it is shown on the basis of agronomic advice that additional N is required to address a proven low protein content in the grain.

Table 5: Available N[1] for barley (kg/ha) where proof of higher yields is available

Soil N Index[2]	Winter Barley[3]		
	8.5 t/ha	**9.5 t/ha**	**10.5 t/ha**
1	180	200	220
2	155	175	195
3	120	140	160
4	80	100	120
	Spring Barley[3,4,5]		
	6.5 t/ha	**7.5 t/ha**	**8.5 t/ha**
1	135	155	175
2	100	120	140
3	75	95	115
4	40	60	80

1. N rates shown above refer to application rates of available fertilizer. Chemical fertilizer rates should be calculated by deducting the available N contained in organic fertilizer applications from the rates shown in the above table.

2. See Tables 6-2 and 6-3 for Soil N Index.

3. Rates shown above are equal to the maximum allowed rates in the NAP regulations. The higher yields shall be based on the best yield achieved in any of the three previous harvests, at 20% moisture content.

4. To reduce the risk of poor establishment in spring cereals, not more than 75 kg/ha N should be combined drilled.

5. Where malting barley is grown under a contract to a purchaser of malting barley an extra 20 kg/ha N may be applied where it is shown on the basis of agronomic advice that additional N is required to address a proven low protein content in the grain.